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ERRATA AND AUTHORS' EMENDATIONS

Page 97, line 20, "were" should read "would be."

Page 110, Table I, column 1, line 13, "24" should read "23."

Page 136, line 2, "*Pseudococcus boninensis*" should read "*Pseudococcus boninsis*."

Page 202, following line 16, add "*C. trifoliata* seedlings, however, on testing are readily susceptible to the disease."

Page 232, Table XVI, footnote, " $r = -0.452 \pm 0.068$ " should read " $r = -0.166 \pm 0.083$."

Page 236, line 1, "The spores, both conidia and ascospores, behaved alike in germination" should read "The conidia from both conidia and ascospores behaved alike in germination."

Page 242, line 22, " $\text{Al}_2(\text{SO}_4)_3 \cdot 0.18 \text{H}_2\text{O}$ " should read " $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$ " and " $\text{FeSO}_4 \cdot 0.7 \text{H}_2\text{O}$ " should read " $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$." The same correction should be made in Tables III, IV, V, and VII.

Page 278, Plate 47 and legend. "Internal hilar sorus shown at x" should be omitted. The marking x is incorrectly placed on the plate. There are two internal hilar sori shown in the lower right-hand portion of the plate.

Page 362, legend for Plate 59, line 10, "Infected leaves from twigs" should read "Infected leaves and twigs."

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A TEOSINTE-MAIZE HYBRID

By G. N. COLLINS, *Botanist*, and J. H. KEMPTON, *Assistant, Acclimitization and Adaptation of Crop Plants and Cotton Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The only plant which has been considered as an ancestor of our cultivated varieties of maize is teosinte (*Euchlaena mexicana* Schrad.). Although placed in a different genus and separated by pronounced morphological differences, teosinte hybridizes freely with maize. In Mexico, where teosinte is native, both teosinte and maize frequently show contamination. Dilute maize hybrids are of such general occurrence in teosinte that it is difficult to decide whether the various forms of teosinte have all descended from one or more wild species.

In attempting to determine more definitely the relation of teosinte to the origin of maize, it is important to know something of the mode of inheritance of the characters which separate the two genera. The following paper is a study of the behavior of a number of the more sharply contrasted characters in the second generation of a hybrid between Florida teosinte and a diminutive variety of maize known as Tom Thumb popcorn. This variety of maize was chosen on account of its very short season and the large number of characters in which it contrasts sharply with teosinte.

The name Florida teosinte is applied to the variety cultivated for forage in the southern part of the United States. This variety shows less evidence of contamination with maize than any other form that has come under our observation, and for this reason it was chosen for these experiments. It is not known how this plant reached Florida. What appears to be the same variety has been obtained from Tampico and Monterey, Mexico, but whether it is native in Mexico has not yet been determined. Seed of the Florida variety has found its way to many tropical countries, and it may have been introduced into eastern Mexico, either directly or indirectly, from Florida. Teosinte is wild in western Mexico; but none of the forms known from that side of the country can with any assurance be referred to the same variety, or even to the same species as the Florida plant.

The restriction of seed production to southern Florida is probably the chief reason why the use of teosinte is not more general, since it is an excellent forage plant. Where comparative tests have been made, it usually produces a larger tonnage of forage than any other plant.

It has been pointed out by Gernert¹ that teosinte is resistant to the attacks of plant lice, an immunity that it would be desirable to transfer to maize. Teosinte also appears to be more resistant to corn smut than any of the varieties of maize with which we are familiar. Stok² reports that in Java teosinte is immune to the chlorosis disease of corn.

Hybrids of maize and teosinte have been grown before, but nothing of commercial importance has thus far been produced. It would seem, however, that if certain combinations of characters could be effected and maintained, the resulting forms would find a place in agriculture.

One of the objects of the experiment was to determine to what extent the characteristics of the parents would be disassociated in the hybrids. Would the much-branched habit of teosinte continue to be associated with a teosinte-like inflorescence, or would profusely branched plants appear bearing maize-like ears? Would the early maturing plants all be maize-like or would there be early plants having the desirable forage characteristics of teosinte?

To proceed with any assurance in securing the desired combinations, it would be of advantage to know to what extent the characters can be separated and with what degree of freedom desirable characters from the different parents can be combined. If, as has been stated,³ hybrids of maize and teosinte eventually revert to either one or the other parent, it would be futile to attempt to secure desirable combinations.

From the standpoint of genetics, the cross is of especial interest, since perhaps nowhere else, with either plants or animals, has it been possible to secure fertile hybrids between two forms separated by such profound structural differences.

FIRST GENERATION OF TEOSINTE-MAIZE HYBRID

Several unsuccessful attempts were made to hybridize the Tom Thumb pop corn and the Florida teosinte in the field, the great disparity in their seasons making it difficult to bring them into flower at the same time. These efforts were continued in the greenhouse, and the hybrids were finally secured in the early spring of 1914.

Because of the peculiar effect of greenhouse conditions, the parental teosinte plants were greatly reduced in size and presented an unusual

¹ GERNERT, W. B. APHIS IMMUNITY OF TEOSINTE-CORN HYBRIDS. *In Science*, n. s. v. 46, no. 1190, p. 390-392. 1917.

² STOK, J. E. VAN DER. BESPREKING DER RESULTATEN VERKREGEN MET DE KRUISING TUSSEN ZEA MAIS L. (MAIS, DJAGOENG) (=REANA LUXURIANS DUR.=TEOSINTE). EN EUCHLAENA MEXICANA SCHRAD. *In Teysmannia*, jaarg. 21, afl. 1, p. 47-59, 1 pl. 1910. Abstract in English in *Amer. Nat.*, v. 47, no. 560, p. 511-512. 1913.

³ HARSHBERGER, J. W. FERTILE CROSSES OF TEOSINTE AND MAIZE. *In Gard. and Forest*, v. 9, no. 462, p. 522-523. 1896. Quotes a letter from Dr. Dugés.

appearance. The plants consisted of single culms not in excess of 50 cm. in height with no suckers and with only from 8 to 11 total nodes. The flowering habits also were affected, the simple culms each terminating in a single spike which produced very little pollen, while the pistillate spikes were borne directly in the axils of the upper two or three sheaths. Accompanying the reduction in size and the alteration in appearance was a corresponding reduction in the time elapsing between germination and flowering. Normal plants grown in Florida flower in about 200 days after germination, while the plants raised in the greenhouse flowered in about 70 days.

The Tom Thumb plants, from seed sown a week or two later, were more nearly normal. Although somewhat reduced in height, the plants produced from 8 to 11 nodes, which is the usual range under field conditions. The terminal inflorescences were entirely staminate, pistillate flowers being produced only in the normal position.

Because of lack of teosinte pollen, all the hybrids were made by using teosinte plants as the female parents. Since the greatest number of seeds in a spike never exceeded 6, the quantity of hybrid seed was small. Three teosinte plants were used as female parents, and a total of 11 hybrid seeds was secured. All these seeds plainly showed the effect of hybridization, being increased in size until they protruded from the hardened glumes.

Nine of the 11 seeds were planted at Lanham in the spring of 1914 and 5 plants reached maturity, though the production of viable seed was prevented by early frosts. Four of the 5 plants were strikingly similar in appearance, and the structure of the inflorescence was alike in all. The fifth plant, though like the preceding 4 in floral characteristics, was greatly reduced in size; in fact, it was little if any taller than normal Tom Thumb but had numerous suckers.

The four normal F_1 plants were about 18 dcm. high with 6 or 7 suckers arising from nodes below the ground. These suckers usually equaled the main stalks in height. In appearance they were replicas of the main culms, though in time of flowering they behaved like those of maize, being several days later. The branching of the main stalk was not continuous, 1 or 2 nodes usually failing to develop branches. These branchless nodes were about the eighth or ninth produced. The total number of nodes on the main culm ranged from 17 to 21. The uppermost branch on three of the plants was in the third node from the top, while the fourth plant was similar to pure teosinte in bearing the uppermost branch at the second node.

The terminal panicles resembled those of maize in that they all had 8-rowed central spikes instead of terminating in a 4-rowed branch as in teosinte; but in three of the four plants this 8-rowed spike drooped as in teosinte, while in maize the central spikes are erect. The pistillate spikes of the hybrid were all 4-rowed, with the spikelets paired and the spikes

decidedly flattened. The plants were much more proterandrous than even normal maize, and the first silks appeared from the basal or prophyllary node of the uppermost branch. About 95 days elapsed from the date of germination before the first pollen was shed, and the first silks appeared from 10 to 21 days later. The season proved to be too short to mature the fruit properly, and no viable seeds were obtained. A photograph of one of the F_1 plants is shown in Plate 6, C, and the pistillate inflorescence of the same plant in Plate 7.

The two seeds remaining from the original cross were planted at Chula Vista, Calif., in 1915, but only one plant was brought to maturity. This plant produced viable seed and became the parent of the second generation discussed in the present paper. Although grown in a climate decidedly different from that at Lanham, Md., the F_1 plant at Chula Vista was strikingly similar in every respect to its sister plants grown the preceding year. It also was proterandrous, though requiring 102 days from germination to the shedding of pollen. The uppermost branch was in the second node from the top, and the plant produced many suckers arising from nodes below the ground. The terminal panicle had an 8-rowed central spike, and the female spikes were all 4-rowed, as in the Lanham plants.

Since the F_1 plants were comparatively uniform, it was not until the great diversity of the second generation became apparent that the characters were formulated. Consequently many of the characters subsequently used were not recorded for the F_1 plants, and no direct comparisons could be made. In any case, the very small number of F_1 plants precluded statistical analysis.

SECOND GENERATION OF TEOSINTE-MAIZE HYBRID

The second generation, consisting of 127 plants, was grown at Chula Vista in the season of 1916. The hills were spaced 4 feet by 3 feet, and only one seed was planted in a hill. This generous spacing, together with the fact that the germination was low, removed all effects of crowding and allowed the plants to develop naturally, an important feature with plants exhibiting such a wide range of size, habits of growth, and season of maturity.

The impression gained from the general appearance of the F_2 plants was that the great majority were of one type, with the remaining plants falling into other fairly well-defined classes. This impression was dispelled as the plants were carefully examined and the measurements of individual characters recorded. The general impression of uniformity was doubtless due to the fact that the branching habit of a plant is its most conspicuous feature. (See Pl. 1.) The measurements showed, in fact, that the number of suckers was among the least variable of the characters measured, 65 per cent of the plants having between 7 and 15 suckers.

METHODS OF MEASUREMENT

The field measurements¹ of the characters, including dates of flowering and size and number of the several organs, were transferred to punched cards, each card representing an individual plant. Practically all the calculations were made by the use of electric sorting and tabulating machines. The distribution and means were obtained by sorting with respect to each character, using the tabulator to count the cards in each class.

In calculating the standard deviation the departures were taken from zero, as recommended by Harris.²

The formula used was $\sigma = \sqrt{\frac{\sum D^2 f}{N} - M^2}$, where σ = standard deviation;

D = departure—in this instance, the class; f = frequency; N = total number; and M = mean. $\sum D^2 f$ was found by multiplying on a calculating machine the summed values for each class (as found by the tabulating machine) by the class and summing the products.

The formula for calculating correlation coefficients proposed by Jennings³ was found to be admirably adapted to the use of tabulating machines.

The formula is

$$r = \frac{\sum XY \cdot N - \sum X \cdot \sum Y}{\sqrt{(\sum X^2 \cdot N - (\sum X)^2) \cdot (\sum Y^2 \cdot N - (\sum Y)^2)}}$$

in which X and Y = the values of the measurements and N = the number of individuals.

In applying this formula the following procedure is recommended by Jennings. Find the values: $\sum X$, $\sum X^2$, $\sum Y$, $\sum Y^2$, and $\sum XY$; next find the values of a , b , and c as follows:

$$a = \sum XY \cdot N - \sum X \cdot \sum Y$$

$$b = \sum X^2 \cdot N - (\sum X)^2$$

$$c = \sum Y^2 \cdot N - (\sum Y)^2$$

Then

$$R_x = \frac{a}{c}$$

$$R_y = \frac{a}{b}$$

and finally $r = \sqrt{R_x \cdot R_y}$.

Since the use of mechanical tabulating machines in the calculation of correlations seems not to have been described, it may not be out of place to explain the procedure followed.

¹ It was necessary to go over the field at intervals of two or three days throughout the growing season to record flowering dates and the position of first silk and to insure an accurate count of the total number of leaves. This work, together with the planting and care of the experiment, was done by Mr. C. G. Marshall.

² HARRIS, J. Arthur. THE ARITHMETIC OF THE PRODUCT MOMENT METHOD OF CALCULATING THE COEFFICIENT OF CORRELATION. *In Amer. Nat.*, v. 44, no. 527, p. 693-699. 1910.

³ JENNINGS, H. S. HEREDITY, VARIATION, AND THE RESULTS OF SELECTION IN THE UNIPARENTAL REPRODUCTION OF *DIPLUGIA CORONA*. *In Genetics*, v. 1, no. 5, p. 407-534, 19 fig. 1916.

The first step in calculating product moment correlations was to reject all cards which did not have values recorded for all the characters. This left a population of 88. The cards were then sorted into the classes of the first character (X of the formula), and the classes were separated by stop cards. While the cards were in this order the tabulator gave the summed values for each of the characters for each class of the first character ($\Sigma_x X$ and $\Sigma_x Y$), the number of individuals in each class, and the total value for each of the characters. Each of the entries in the table thus formed ($\Sigma_x X$ and $\Sigma_x Y$) was then multiplied by the class value, and the products were summed on a calculator, giving ΣX^2 and ΣXY .

These summations when multiplied by the number gave $\Sigma X^2 \cdot N$ for the first character and $\Sigma XY \cdot N$ for the remaining characters in the formula for all correlations with the first character. The totals for each character multiplied by the total of the first character gave $(\Sigma X)^2$ for the first character and $\Sigma X \cdot \Sigma Y$ for the remaining characters.

The cards were then sorted for the second character, and the same procedure was followed. In each operation the totals should check, and since each character entered as both X and Y , no additional checking is necessary, each correlation being in effect calculated twice with each operation independently checked. The actual regression lines were readily plotted by dividing the values ΣYX by the number of individuals in the respective classes.

The number of characters for which all correlations can be calculated is limited, of course, by the number that can be recorded on a card. The largest card at our disposal had 45 columns, which would accommodate but 26 characters; and since we wished to consider 33 characters, a second card was used on which the more important characters were repeated, with the addition of the characters not recorded on the first card.

The distributions in the alicole group were bimodal to an extent that seemed to preclude the use of the product-moment method. Correlations within this group were, therefore, calculated by Yule's method for the coefficient of association. Biserial correlations were used to determine the relation between alicole characters and characters outside this group.

Probable errors are not given in the table, since all correlations were calculated from the same population of 88 individuals. In the discussion, correlations of less than 0.25, which is 3.5 times the error, are considered insignificant.

In discussions of genetic correlations it is necessary to distinguish between the instances where two characters derived from the same parent tend to be inherited together and those where one of the characters has entered the hybrid from one parent and the correlated character has been derived from the other parent.

The terms "coherence" and "disherence" will here be used to designate the direction of the correlations with respect to the parental combinations.

The terms "linkage" or "coupling," which are in more general use, might be used in place of coherence; but both these terms imply that the relation is between Mendelian or alternative characters, while most of the characters under discussion show quantitative instead of alternative differences. Furthermore, there appears to be no term in general use that can be applied to the cases where the correlation is the opposite of a linkage or coupling. The use of the word "repulsion" would seriously confuse the issue, since that term implies the disassociation of dominant characters without regard to whether they have entered the hybrid from the same or different parents. In using "coherence" instead of "linkage" there is no intention to imply that the ultimate determinants of the characters are not inherited in Mendelian fashion; but since no attempt toward factorial analysis is made, it seems better to use a more general term.

DESCRIPTION OF CHARACTERS

Thirty-three characters were recorded and their correlations considered. Many of these characters fall into groups the members of which would seem to be mutually related, either physically or physiologically. Eight such groups are recognized, comprising in all 26 characters. Among the 7 remaining characters considered as independent, physiological relations, if they exist, are more obscure. The grouping of the characters is shown below, with the abbreviated designations of the characters which will be used throughout the paper.

HEIGHT GROUP (P. 11-16)

HEIGHT.—Height of the main culm in decimeters.

TOTAL LEAVES.—Total number of leaves or nodes produced on the main culm.

HEIGHT OF SUCKER.—Height of the tallest sucker or tiller in decimeters.

SUCKER INDEX.—Height of the tallest sucker, expressed as a percentage of the height of the main culm.

CIRCUMFERENCE INDEX.—Circumference of the thickest internode in millimeters, expressed as a percentage of the height of the main culm measured in centimeters.

NODES WITHOUT BRANCHES.—Number of nodes between the uppermost sucker, or the surface of the ground, and the lowest developed branch.

NODES ABOVE GROUP (P. 16-19)

NODES ABOVE.—Number of nodes on the main culm above the ear or uppermost branch.

NODES ABOVE ON THIRD.—Number of nodes above the uppermost secondary branch of the third branch from the top.

NODES ON THIRD.—Number of nodes on the third primary branch from the top.

TASSEL GROUP (P. 19-21)

PRIMARY BRANCHES.—Number of primary branches in the terminal inflorescence of the main culm.

SECONDARY BRANCHES.—Number of secondary branches in the terminal inflorescence of the main culm.

SECONDARY INDEX.—Number of secondary branches, expressed as a percentage of the primary and secondary branches combined.

TASSEL BRANCHES ON THIRD.—Number of branches in the terminal inflorescence of the third branch from the top.

MALE BRANCH GROUP (P. 21-22)

MALE BRANCH INDEX.—Number of primary branches terminating in a staminate inflorescence, expressed as a percentage of the total leaves.

MALE SECONDARIES.—Number of secondary branches terminating in a staminate inflorescence on the third branch from the top.

ALICOLE GROUP (P. 22-25)

DOUBLE MALE ALICOLES.—Number of alicoles or alveoli with two staminate spikelets in the best-developed spike of the pistillate inflorescence, expressed as a percentage of the total number of alicoles in the spike.

MIXED ALICOLES.—Number of alicoles with one staminate and one pistillate spikelet in the best-developed spike, expressed as a percentage of the total number of alicoles in the spike.

SINGLE FEMALE ALICOLES.—Number of alicoles with a single pistillate spikelet in the best-developed spike, expressed as a percentage of the total number of alicoles in the spike.

DOUBLE FEMALE ALICOLES.—Number of alicoles with two pistillate spikelets in the best-developed spike, expressed as a percentage of the total number of alicoles in the spike.

ALICOLE INDEX.—Number of alicoles with a single pistillate spikelet, expressed as a percentage of the sum of single and double female alicoles in the spike.

NODES SILKING GROUP (P. 25-26)

NODES SILKING ON THIRD.—Number of nodes producing silks on third branch from top.

NODES SILKING INDEX.—Number of nodes producing silks on third branch, expressed as a percentage of the number of nodes on the third branch.

PROPHYLLARY GROUP (P. 26-27)

PROPHYLLARY SPIKES.—Number of pistillate spikes in the axil of the prophyllum of the third branch.

LENGTH OF PROPHYLLARY.—Length in centimeters of the prophyllary branch of the third branch from the top.

NUMBER OF ROWS GROUP (P. 27-28)

ROWS IN CENTRAL SPIKE.—Number of rows of spikelets in the central spike of the terminal inflorescence of the main culm.

ROWS OF ALICOLES.—Number of rows of alicoles in the best-developed pistillate spike of the third branch from the top.

INDEPENDENT CHARACTERS (P. 23-33)

POSITION OF BEST SPIKE.—Position on the third branch of the node bearing the best-developed spike. The nodes were numbered from the base of the branch, the branch in the axil of the prophyllum being recorded as zero.

NUMBER OF ALICOLES.—Number of alicoles in the best-developed spike of the third branch from the top.

NUMBER OF SUCKERS.—Number of branches on the main culm or on primary branches that originated below or near the surface of the ground.

BRANCH SILKING FIRST.—Number of branches on the main culm above the branch on which silk appeared earliest.

DAYS TO POLLEN.—Number of days from planting to the first production of pollen.

POLLEN TO SILK.—Number of days from the first production of pollen to the first emergence of silks.

LENGTH OF INTERNODE ON THIRD.—Length of the third branch from the top divided by the number of internodes on the same branch.

The reasons for the grouping of the characters are in most instances obvious. A discussion of the less obvious relationships will be found under the descriptions of the various characters.

All measurements of characters pertaining to the pistillate inflorescence were taken on the third branch from the top of the plant. Some limitation of this kind was necessary to simplify the comparisons, and this branch was chosen as representing the region of maximum development of seed. Reference to Tables I and II shows that in pure teosinte this is the branch with the largest spikes and the largest number of seeds per node.

TABLE I.—Number of spikes at each node of the various branches of a plant of *Florida teosinte*

[illegible]

TABLE II.—Number of seeds at each node of the various branches of a plant of *Florida teosinte*

[illegible]

A knowledge of the behavior of the individual characters in the second generation can best be obtained by a study of the distribution diagrams, figures 1 to 33.

To facilitate the study of the relation of the characters to one another in inheritance, the table of correlations, Table III, is provided. Anything approaching a complete analysis of the data is, of course, out of the question; but the correlation coefficients and the statistical constants given in Tables IV and V afford a means for testing the validity of any assumed relationship. In the discussion of the characters an attempt will be made to indicate the more striking correlations.

TABLE IV.—Distribution of individuals in F_2 of teosinte-maize hybrid with respect to various characters

Units of measurement.	Height (fig. 1). ^a	Total leaves (fig. 2). ^a	Height of sucker (fig. 3). ^a	Nodes without branches (fig. 6). ^a	Nodes above (fig. 7). ^a	Nodes above on third (fig. 8). ^a	Nodes on third (fig. 9). ^a	Primary branches (fig. 10). ^a	Secondary branches (fig. 11). ^a	Tassel branches on third (fig. 13). ^a	Male secondaries (fig. 15). ^a	Nodes silking on third (fig. 21). ^a
0.....				79	1				2	7	56	1
1.....				4	85	47			1	10	10	
2.....	1			9	34	61			3	9	10	
3.....	1			12	3	10			6	11	13	8
4.....	1			5	2	3	5		11	11	6	27
5.....				3			22	1	4	7	12	26
6.....			1	2		1	29		8	9	10	25
7.....			1	2			24		9	8	4	21
8.....	5			2			20		10	10	1	9
9.....	3	1	1	1			7	4	8	1		3
10.....	6		2	1			12	4	7	13		1
11.....	5		4				2	3	9	2		
12.....	25		12				1	5	1	5		
13.....	4	1	7					11	7	1		
14.....	21	1	11				1	9		2		
15.....	9	1	8					10	2	3		
16.....	11	4	21					11	4	2		
17.....	9	11	12					12	2			
18.....	8	6	14					14	3	1		
19.....	5	10	7					9	2			
20.....	3	7	10					5	4			
21.....	1	19	5					11				
22.....	4	3	4					5	4			
23.....	1	11						5				
24.....		6	1					3	1			
25.....		8	1					1	2			
26.....		5							1			
27.....		2	1									
28.....		7						1	2			
29.....		3						1	1			
30.....		6										
31.....		3										
32.....		1										
33.....		5							3			
34.....									1			
35.....												
36.....									1			
37.....												
38.....									1			
39.....		1										
40.....									1			
41.....												
42.....									1			
44.....												
46.....												
52.....									1			
53.....												
Number.....	123	122	123	120	125	122	123	125	125	112	122	121
Mean.....	14.1	22.7	16.2	1.28	1.36	1.78	7.0	16.8	10.9	6.13	2.04	5.52
Standard deviation.....	4.0	5.1	3.47	2.21	.62	.81	1.92	4.1	11.8	4.07	2.39	1.67

^a Figures indicate number of plants exhibiting each character to the extent shown in the first column. For discussion of units of measurement see p. 7-8.

Desig- ning ex.	Prophyl- lary spikes.	Length of pro- phyllary.	Rows in central spike.	Position of best spike.	Number of alicoles.	Number of suckers.	Branch silking first.	Days to pollen.	Pollen to silk.	Length of inter- node on third.
0.46	0.07	0.16	D 0.05	-0.20	D 0.07	0.05	0.02	0.47	D 0.11	D-0.33
.12	.01	D- .04	- .14	- .18	- .18	D- .10	.33	.79	D .02	D- .66
.02	D- .01	.20	D .06	D .03	D .17	.21	.03	.1402
.23	D- .12	.01	D- .01	D .31	D .09	.17	D- .36	D- .42	D .18	.48
....	.00	D .02	D- .03	.21	.12	- .19	- .17	.31
.07	- .17	- .17	.02	D- .15	D- .12	- .31	D .04	D .36	- .29
.17	- .13	- .09	.20	.10	.20	- .15	- .30	- .23	D- .06	D .04
.38	- .10	- .06	.26	.03	.16	- .02	- .26	- .26	D- .04	- .02
.22	- .29	- .19	.05	.73	.15	D .05	- .37	.11	- .05
.12	.03	.04	D .12	- .02	D .10	.01	.01	.32	D .04	D- .25
.14	.05	- .00	- .07	- .20	- .08	D- .01	.32	.59	D- .36
....	.00	.02	- .30	- .17	- .25	.0457	- .03
.20	D- .09	D- .16	- .18	D .24	- .19	D- .05	.05	.17	D- .15
.02	.23	.12	- .04	.12	D .06	.14	.33	.03	D- .04
.13	.19	.38	D .08	D .18	D .16	.02	D- .29	D- .40	D .04	.51
....	D- .13	.10	D .22	D .03	D .13	.0000	.00
....	D- .09	.12	- .29	- .08	- .15	.0000	.00
....	D- .04	D- .10	- .35	.00	- .38	.3015	D .01
....	D .07	D .13	.23	.04	.31	- .29	- .15	.05
.10	D- .09	D- .10	- .12	- .05	- .37	.11	.09	.17	D- .21
.35	.00	.09	- .03	D .47	D .05	.01	D- .12	.07	D .18	D- .04
....	.39	.40	- .04	- .25	D .09	.03	.07	.0609
.3959	D .08	- .56	D .05	.08	.14	.08	- .18	.10
.40	.59	- .01	D .31	D .15	.10	.05	D- .19	D .06	.29
.04	D- .08	- .01	D- .05	.37	D .02	- .01	- .09	D- .18	D .06
.25	- .56	D .31	D- .0501	D .09	- .34	- .09	.40	D .02
.09	D .05	D .13	.37	.01	- .11	D .05	- .29	.01	D .23
.03	.08	.10	D .02	D .09	- .1102	D- .09	- .23	.16
.07	.14	.05	- .01	- .34	D .05	.0231	D- .21
.06	.08	D- .19	- .09	- .09	- .29	D- .09	.31	- .14	D- .56
....	- .18	D .06	D- .18	.40	.01	- .23	- .14
.09	.10	.29	D .06	D .02	D .23	.16	D- .21	D- .56

TABLE IV.—Distribution of individuals in F_2 of teosinte-maize hybrid with respect to various characters—Continued

Units of measurement.	Prophyllary spikes (fig. 23). ^a	Length of prophyllary (fig. 24). ^a	Rows in central spike (fig. 25). ^a	Rows of all-coles (fig. 26). ^a	Position of best spike (fig. 27). ^a	Number of all-coles (fig. 28). ^a	Number of suckers (fig. 29). ^a	Branch silking first (fig. 30). ^a	Days to pollen (fig. 31). ^{a, b}	Pollen to silk (fig. 32). ^a	Length of internode on third (fig. 33). ^a
0.....	23	13			22		3		3	1	
1.....	23				23			43	15	2	
2.....	12			III	18		1	43	27	1	2
3.....	12			10	29		2	11	25		1
4.....	10		23	I	11		8	6	15	1	6
5.....	11	3	14		6		2	3	13	1	4
6.....	8		19	I	5		3		11	5	5
7.....	5		5		3	1	11		10	4	5
8.....	1	3	62		1	1	8		3	4	14
9.....	2	7			2	3	10		3	3	5
10.....	3	9	2			1	8			8	15
11.....	3	5				1	10			5	8
12.....		15				5	9			3	17
13.....		15				9	11			5	9
14.....	1	8				9	9			1	6
15.....		9				7	7			3	6
16.....		7				16	2			8	3
17.....		4				6	1			7	5
18.....		4				16	5			6	3
19.....		2				14	3			6	
20.....		2				8	5			8	4
21.....		1				4	2			2	
22.....		2				2	3			1	1
23.....		3				5	1			6	
24.....		2								3	
25.....		1				2				4	
26.....						4	1			2	
27.....		1					1			4	
28.....						1				3	
29.....						1				1	
30.....						3				1	
31.....											
32.....		2					1			1	
33.....										1	
34.....						2				1	
35.....										3	
36.....							1			1	
37.....		1								2	
38.....											
39.....										1	
40.....		1									
41.....						2					
42.....		1									
44.....										1	
46.....											
52.....										1	
53.....										1	
Number.....	114	121	125	123	120	123	127	106	125	122	119
Mean.....	19	13.4	6.62	2.13	2.47	17.9	11.7	1.89	111.9	18.3	10.9
Standard deviation.....	3.04	7.69	1.87	.38	2.14	6.17	3.36	.44	21.5	9.7	4.19

^a Figures indicate number of plants exhibiting each character to the extent shown in the first column. For discussion of units of measurement see p. 7-8.
^b First date recorded 71 days after planting and subsequently at 10-day periods.

DISCUSSION OF CHARACTERS AND THEIR CORRELATIONS

HEIGHT GROUP

HEIGHT

Confining the measurement of height to the main stalk does not always give a fair idea of the size of the plant, since there were many individuals in which the suckers greatly exceeded the main stalk. (See distribution of sucker index, Table V.)

TABLE V.—Distribution of individuals in F_2 of teosinte-maize hybrid with respect to characters recorded as indices

Units of measure- ment.	Double male ali- coles (fig. 16). ^a	Mixed alicoles (fig. 17). ^a	Single female ali- coles (fig. 18). ^a	Double female alicoles (fig. 19). ^a	Alicole index (fig. 20). ^a	Units of measure- ment.	Sucker index (fig. 4). ^a	Units of measure- ment.	Circumference in- dex (fig. 5). ^a	Units of measure- ment.	Secondary index (fig. 12). ^a	Units of measure- ment.	Male branch in- dex (fig. 14). ^a	Nodes silking in- dex (fig. 22). ^a
Per ct.						Per cent.		Per ct.		Per cent.		Per ct.		
0-4	105	110	34	18	36	41-50	1	24	1	0	2	0-9	1	1
5-14	2	6	27	4	25	51-60	2	25	1-10	1	10-19	2
15-24	3	1	13	6	12	61-70	1	26	1	11-20	13	20-29	26
25-34	6	3	5	4	5	71-80	1	27	21-30	12	30-39	23	1
35-44	5	3	5	8	5	81-90	11	28	1	31-40	17	40-49	48	2
45-54	2	4	7	3	91-100	20	29	1	41-50	12	50-59	15	8
55-64	7	9	7	101-110	30	30	51-60	12	60-69	5	11
65-74	5	6	4	111-120	21	31	2	61-70	12	70-79	4	15
75-84	6	14	4	121-130	19	32	1	71-80	9	80-89	27
85-94	5	23	3	131-140	7	33	4	81-90	8	90-99	21
95-100	12	24	19	141-150	2	34	1	91-100	1	100	35
.....	151-160	1	35	3	101-110	7
.....	161-170	36	3	111-121	4
.....	171-180	1	37	2	121-130	3
.....	38	5	131-140	1
.....	221-230	1	39	4	141-150	2
.....	40	8	151-160	2
.....	451-460	1	41	7	161-170
.....	42	5	171-180	1
.....	43	5	181-190	3
.....	44	6	191-200	1
.....	45	5
.....	46	4	321-330	1
.....	47	4
.....	48	5
.....	49	3
.....	50	4
.....	51
.....	52	1
.....	53	2
.....	54	4
.....	55	3
.....	56	1
.....	57	4
.....	58	2
.....	61	1
.....	62	1
.....	63	2
.....	69	1
.....	78	1
.....	83	1
.....	91	1
No..	123	123	123	123	123	119	110	124	118	121
M...	4.55	2.36	32.4	61.0	34.0	121.5	45.2	70.0	41.5	80.6
σ.....	11.9	7.96	46.5	68.0	41.3	37.7	12.4	49.0	8.8	18.8

^a Figures give number of plants exhibiting each character to the extent shown in first column in this section of the table.

The average height of Tom Thumb maize plants at Chula Vista was 6 dcm. and that of Florida teosinte 23 dcm. The F_1 plants averaged 17 dcm. The mean of the F_2 plants was 14. The range was from 2 to 23 dcm. The distribution (fig. 1) was as nearly normal as could be expected from the number of individuals involved. There is, furthermore, no indication of skewness, the mode and the mean practically coinciding.

Although the parental varieties differ greatly in height, the parental species overlap. Indeed the taller varieties of maize probably exceed the tallest teosinte in height.

Height is positively correlated with all of the four tassel measurements, and the correlations are significantly higher than was found in a progeny

of Tom Thumb where two of these characters were recorded. Thus there is evidence of coherence between height and the character of the tassel.

Disherence with male secondaries is indicated by a correlation of -0.28 ± 0.07 . The negative correlation of -0.46 ± 0.06 with nodes silking index would also seem a clear example of disherence.

The correlation of 0.47 with days to pollen indicates coherence of this character with height. In both parent populations this correlation was negative, but under most circumstances the late plants of a maize variety are taller than the early plants.

The negative correlation of -0.33 with length of internode on third is in the direction of a disherence, though this is probably associated with the negative correlation between height and sucker index, which is to some extent physical. Anything which tended to interfere with the growth of the main culm would doubtless stimulate the development of all the branches.

TOTAL LEAVES

The total number of leaves on the main culm in Tom Thumb is usually 11, in Florida teosinte about 37. The mean in the F_2 hybrid plants was 23, with a range from 9 to 38. The distribution (fig. 2) is normal, and the variability

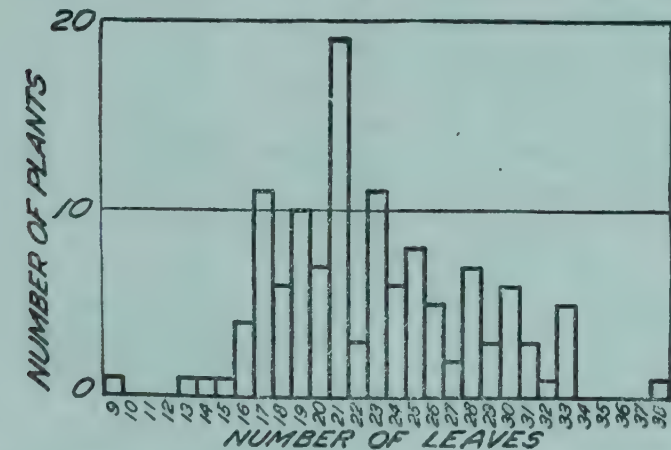


FIG. 2.—Total leaves: frequency distribution of plants in F_2 . Class value, one leaf.

as measured by the coefficient of variation is the lowest recorded for any character. usually an intra-variatal correlation of about 0.3 between total leaves and height. Corresponding data for teosinte are not available, but the coefficient of 0.69 in the hybrid material affords some evidence of coherence between these characters.

The correlations of total leaves with other characters are similar to those of height, with the exception that there is no evidence of disherence with nodes silking index. There is also coherence with branch silking first.

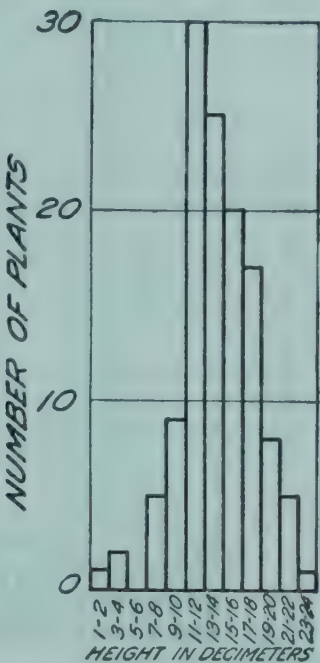


FIG. 1.—Height: frequency distribution of plants in F_2 . Class value, 2 dcm.

as measured by the coefficient of variation is the lowest recorded for any character.

The larger varieties of maize equal or exceed teosinte in number of leaves just as they do in height. In both maize and teosinte total number of leaves is a character very little affected by changes in the environment. In maize there is

HEIGHT OF SUCKER

Measurements were taken from the ground to the tip of the tassel of the tallest sucker or tiller and recorded in decimeters. Tom Thumb almost never produces a sucker. In Florida teosinte there are usually numerous

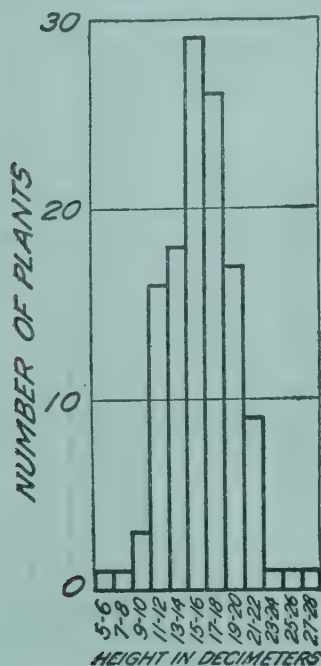


FIG. 3.—Height of sucker: frequency distribution of plants in F_2 . Class value, 2 dcm.

culm. This measurement was taken as the best single expression of the tendency to produce tall suckers. Since Tom Thumb almost never produces suckers, the index is practically zero for the male parent of the hybrid. In Florida teosinte the index is usually about 100. In one population of 87, the mean was 99.4, with a range from 90 to 110. In the F_2 hybrid plants the mean was 117, with a range from 50 to 460. The distribution (fig. 4) was unimodal and symmetrical with the exception of a few stragglers probably representing plants with abnormal main culms.

The coherences outside the group are with male secondaries, mixed alicoles, and length of internode on third. The disherences are with three members of the height group, nodes on third branch, two of the tassel measurements, position of best spike, branch silking first, and days to pollen.

There is thus more direct evidence of disherence than of coherence with this character. It should be remembered, however, that the negative correlation of sucker index with height is in a sense physical, since the

suckers of practically the same height as the main culm. The parent varieties are thus widely separated, but there are varieties of maize with suckers taller than any recorded in teosinte. The mean of the F_2 hybrid plants was 16.2, ranging from 6 to 27, with a practically normal distribution (fig. 3).

The only character outside the group showing a significant correlation with height of sucker is secondary branches. The correlation is in the direction of a coherence.

SUCKER INDEX

This character was determined by dividing the height of the tallest sucker by the height of the plant and multiplying by 100. It is thus the height of the tallest sucker expressed as a percentage of the height of the main

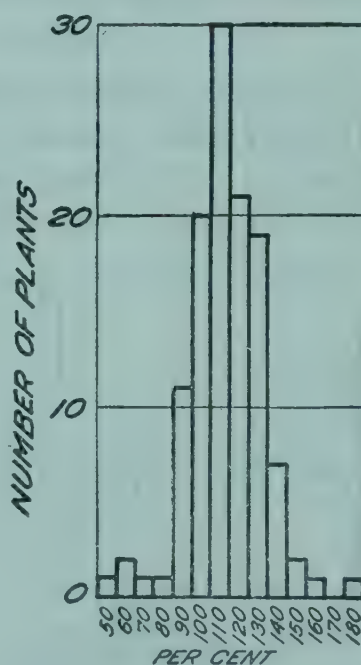


FIG. 4.—Sucker index: frequency distribution of plants in F_2 . Class value, 10 per cent. One plant at 230 and one at 460.

one is a function of the other. The other disherences may follow as secondary relations due to this correlation with height.

CIRCUMFERENCE INDEX

In a population of 87, the circumference of the culm of Florida teosinte averaged 61 mm. Under similar conditions the circumference of Tom Thumb was approximately 35 mm. The mean of the F₂ hybrid plants was 56 mm.

Since circumference is so closely associated with the general size of the plant, the circumference measurement was recorded as a percentage of the height of the plant, and the measure- . ment is termed a circumference index.

While in direct measurement the culms of teosinte are thicker than those of Tom Thumb, teosinte is much more slender. In circumference index a high value is therefore a variation toward the maize parent. The mean index of Florida teosinte was 2.7, that of Tom Thumb about 6.0. The mean of the hybrid plants was 4.5, with a normal distribution (fig. 5).

Circumference shows one significant and independent coherence, that with pollen to silk, and a disherence with male secondaries.

NODES WITHOUT BRANCHES

This character is the number of nodes between the lowest branch and the uppermost sucker or the surface of the ground. In teosinte, branches are normally developed in the axils of all leaves on the main culm, except the uppermost. The tendency to suppress branches at the nodes just above the ground appears, however, when the plants are grown under unfavorable conditions. In a planting of Florida teosinte at Chula Vista in 1918 the average number of nodes without branches was 7.6.

In maize there are always a number of nodes without branches between the uppermost sucker and the lowest ear. In Tom Thumb where no suckers are developed, the number can not be definitely determined, since the surface of the ground can not be located with accuracy. But since the average total number of leaves in Tom Thumb is 11 and there is an average of 3 nodes above the single ear and about 5 nodes below the surface of the ground, the mean number of nodes without branches is about 3.

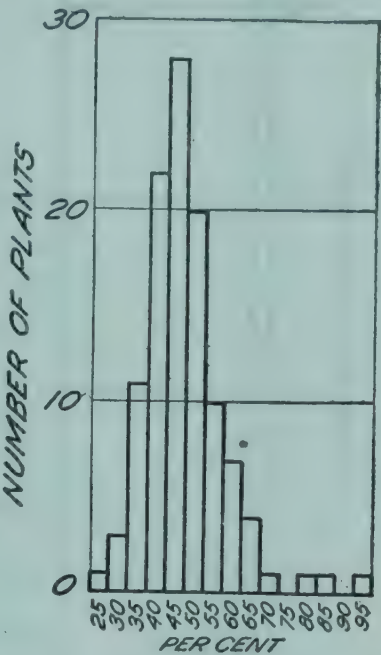


FIG. 5.—Circumference index: frequency distribution of plants in F₂. Class value, 5 per cent.

The mean number of nodes without branches in the F_2 hybrid plants was 1.05. The distribution (fig. 6) was far from normal, and there is some indication of two modes. Seventy-nine of the individuals were at zero. Of the remaining 41 plants the largest number, 12, had 3 nodes without branches, with a fairly uniform distribution ranging from 1 to 9.

The significant correlations outside the group were coherences with both of the characters in the male branch group, number of suckers and length of internode on third. The differences are with secondary branches and days to pollen.

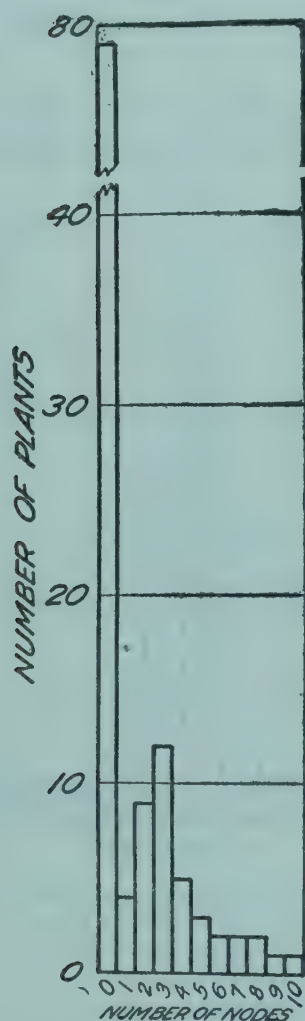


FIG. 6.—Nodes without branches: frequency distribution of plants in F_2 . Class value, one node.

NODES ABOVE GROUP

NODES ABOVE

In teosinte of all varieties there is almost without exception one node above the uppermost branch. In maize the number varies from 8 or 9 to 2 or 3; only in rare and abnormal specimens is it reduced to one. The limits as observed in the Tom Thumb variety are 3 and 5, with the mean at 3.4. This character, while not so constant as total leaves, is less subject to environmental influences than most of the characters recorded.

There is some question of the propriety of considering the number of nodes above the ear in maize as strictly homologous with the number of nodes above the uppermost branch of teosinte. In maize the uppermost branch, or ear, is normally the best developed, while in teosinte the most fruitful branch is usually the third or fourth from the top. See Tables I and II.

If the uppermost branch in teosinte is not homologous with the uppermost branch or ear in maize, the complete absence of any trace of a bud in the axils of the leaves above the upper ear in maize calls for some explanation. It is difficult to believe that branches in the axils of the upper leaves of maize could have been so completely suppressed as to leave neither a trace nor a tendency to reappear as an abnormality. It appears more reasonable to assume that in maize additional nodes have been intercalated or that these sterile nodes in maize, instead of representing a change from the condition found in teosinte, have been derived from a distinct ancestor.

In the first generation there were two plants with one node above and three with two. The range in the second generation was from one to four, with one possibly abnormal plant with none. The distribution

(fig. 7) is decidedly skew, more than half the plants having one, but there is no indication of bimodality.

In maize there is always an intravarietal correlation between nodes above and total number of leaves and other characters that are expressions of size. Since Tom Thumb maize is smaller and has a much smaller number of leaves than teosinte, coherence with these size characters would not be masked by physiological correlations.

It is therefore interesting to note that the tassel characters, which in pure strains of maize are positively correlated with nodes above, are here negatively correlated, affording clear evidence of coherence. There are also significant correlations in the direction of coherence with the male branch characters and node silking first. There are no significant disherences.

NODES ABOVE ON THIRD

In all varieties of teosinte the number of nodes above the uppermost secondary on the third branch is one, as on the main culm. In maize the value will depend on what is considered the homologue of the third branch from the top in teosinte. Taken strictly, the upper branch in maize is the ear, and the third branch from the top, when such exists, would be an earlike branch that in some types would partake somewhat of the nature of a sucker. If sufficiently suckerlike, the number of nodes above the uppermost secondary of such a branch would correspond to those of the main stalk, that is, the range would be from 3 to 8. If, however, the ear of maize be assumed to correspond to some branch below the uppermost in teosinte, those above the ear having been suppressed, the number of nodes above the uppermost secondary would be much greater, for in this case the branch would be an ear and the secondary branches would be the secondary ears which almost invariably are borne in the axil of the lowest husk. In any case the number would be larger in maize than in teosinte.

This character was recorded for three of the F_1 plants. In two of these the number was 1; in the other it was 2. The average number in the F_2 hybrid plants was 1.78, with no indication of bimodality. The distribution (fig. 8) is much less skew than for the nodes above on the main culm, the mode being at 2. In its correlations, this character is similar to nodes above on the main stalk.

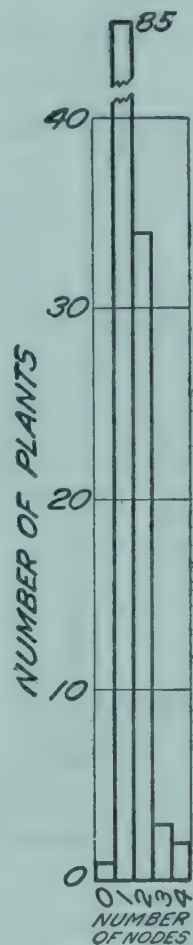


FIG. 7.—Nodes above: frequency distribution of plants in F_2 . Class value, one node.

NODES ON THIRD

This character is instructive chiefly as a means of throwing light on the homologies between the branches of teosinte and maize and as a means of calculating the average length of internodes on the third branch described below.

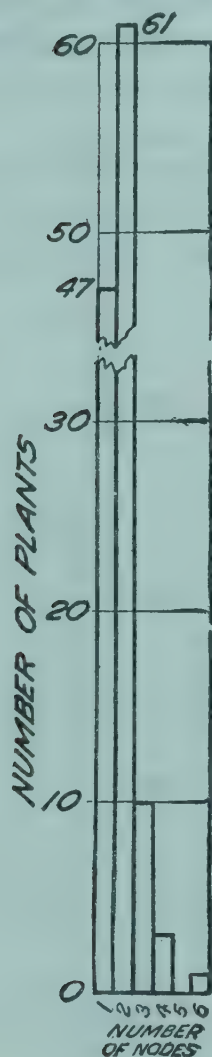


FIG. 8.—Nodes above on third: frequency distribution of plants in F_2 . Class value, one node.

If each husk on an ear of maize represents a node, the third branch from the top, which would still be earlike, would have even in Tom Thumb from 6 to 8 nodes. In other varieties this number would be even larger. On the other hand, if the leaves from which the husks are derived have been subdivided, thus increasing the apparent number of nodes, the number of nodes on this branch of the hybrids might be expected to agree pretty closely with the number in teosinte, which varies from 2 to 5. The modal number in the F_2 hybrid plants was 6, the mean was 7.15, with a range from 4 to 14. There was no indication of bimodality (fig. 9). There was no indication in the hybrid plants that leaves were subdivided, each leaf being borne on a well-defined internode. The increased number of nodes over that of teosinte goes to support the idea that each of the husks on an ear of maize represents an internode of the branch.

In common with the other characters of this group, the correlations with secondary branches and prophyllary spikes would seem significant coherences. In these correlations a high value of one character is correlated with a low value of the other, and any general tendency

to vigor would reduce the correlation.

The correlations with position of best spike and node silking first are of doubtful significance, there being an obvious physiological connection between these characters and the number of nodes in the third branch.

All the significant disherent correlations are of a nature that suggests a physiological explanation.

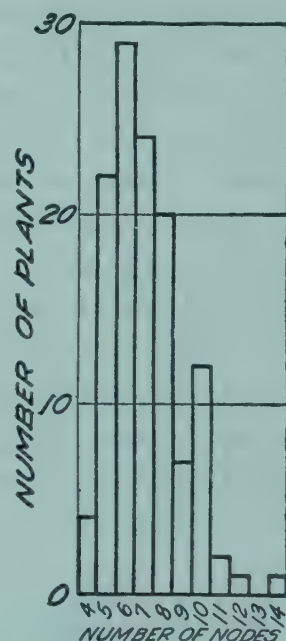


FIG. 9.—Nodes on third: frequency distribution of plants in F_2 . Class value, one node.

TASSEL GROUP

PRIMARY BRANCHES

Primary tassel branches are much more numerous in teosinte than in any but the very largest varieties of maize. In the Tom Thumb variety the maximum number observed was 9, and this falls far below the number in any normal teosinte plant. The mean number for Tom Thumb and Florida teosinte grown under similar conditions was 4.6 and 12.5, respectively. In the F_1 plants the number ranged from 16 to 20. In the second generation the mean was 16.7 with a range from 5 to 29. The distribution (fig. 10) was symmetrical and unimodal.

There are two significant independent coherences, one with characters of the height group and the other with days to pollen. There are also two disifferences, one with number of single female alicoles, the other with length of internode on third. The apparent disifference with sucker index is probably associated with the negative correlation of sucker index with the other height characters.

SECONDARY BRANCHES

Teosinte has a much larger number of secondary tassel branches than maize. The specific ranges of the parents may overlap, but the Tom Thumb variety seldom develops secondary branches, while in Florida teosinte the mean number was 10.3.

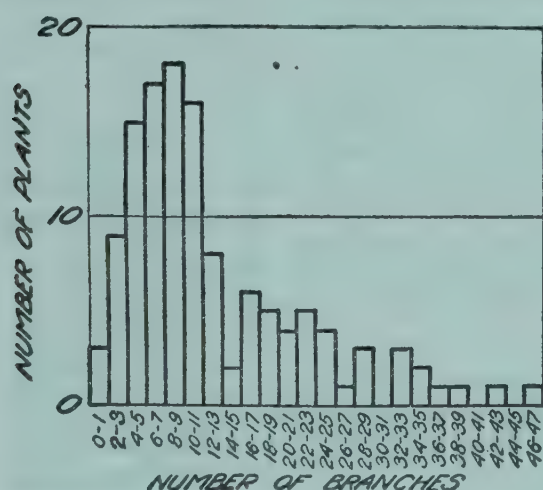


FIG. 11.—Secondary branches: frequency distribution of plants in F_2 . Class value, two branches.

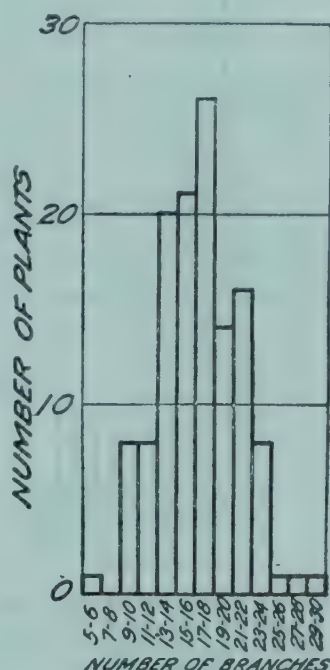


FIG. 10.—Primary branches: frequency distribution of plants in F_2 . Class value, two branches.

In the F_2 hybrid plants the mean was 12.6, with a range from 0 to 46. The distribution (fig. 11) is very skew, the mode being near 8, but there is little evidence of more than one mode.

This character shows more evidence of coherence than does the character primary branches. It is closely correlated with three of the measurements of height.

There is the same negative correlation with sucker index; and in addition the positive correlation with nodes without branches, which is in the direction of a disifference, is here above 0.25.

A most striking example of coherence is the negative correlation of secondary branches with all three of the nodes above group. The other coherences are with male branches, branch silking first, and days to pollen. The only clear evidence of disherence is with male secondaries and length of internode on third.

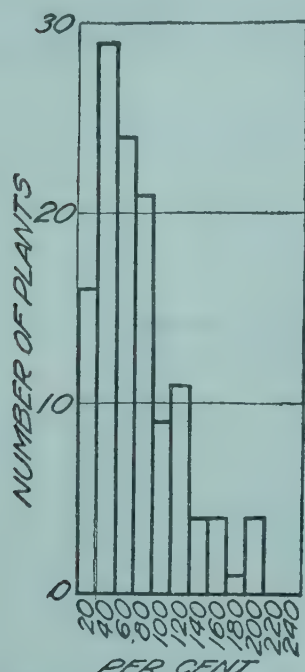


FIG. 12.—Secondary index: frequency distribution of plants in F_2 . Class value, 20 per cent. One plant at 340.

SECONDARY INDEX

This character, which is the number of secondary branches expressed as a percentage of the total branches, distinguishes more sharply between maize and teosinte than does the direct measurement of either primary or secondary branches. Secondary tassel branches are relatively as well as absolutely much more numerous in teosinte than in maize. In teosinte they equal or exceed the number of primary branches, while in maize the number of secondaries equals the number of the primaries only in some of the large tropical varieties.

In the F_2 hybrid plants the mean was 70, with a very skew distribution (fig. 12) but with no evidence of more than one mode.

The correlations are similar to those with the direct measurements of tassel branches, except the additional coherences with number of alicoles and rows in central spike.

TASSEL BRANCHES ON THIRD

In teosinte the modal number of tassel branches on the third branch from the top is two. When teosinte is grown under rather unfavorable conditions where the number of branches is reduced, there is evidence of a bimodal distribution, in that plants with two branches or none are more numerous than plants with a single branch. In maize the number is zero, since all branches from the upper nodes of maize are normally unbranched.

In the F_2 hybrid plants the mean was 6.1. The distribution (fig. 13) was skew, with slight indication of two modes.

Although closely correlated with the tassel characters of the main stalk, this character shows no significant correlations outside the group except with number of nodes on third.

This is in the direction of a disherence; but the relation is doubtless physiological, since both characters would be similarly affected by changes in general vigor.

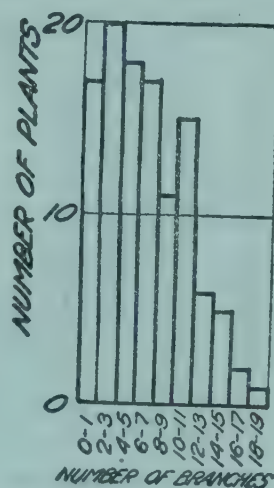


FIG. 13.—Tassel branches on third: frequency distribution of plants in F_2 . Class value, two branches.

The five tassel characters form a closely correlated group. With few exceptions all the members of the group show similar relations with other characters. With the exception of secondary index, all are direct measurements that might be expected to increase with increased vigor; and were it not for this character the correlations with the direct measurements in the height group might be considered physiological. The same may be said of the nodes above group. The disherent correlation of tassel branches on third with nodes on third branch is also physiological, since a highly developed third branch would naturally have a larger number of tassel branches.

The clearest evidences of coherence are the correlations of secondary index with rows in central spike and that between secondary branches and branch silking first. Disherence is indicated by the negative correlations between all the tassel characters and the two characters male secondaries and length of internode on third.

MALE BRANCH GROUP

MALE BRANCH INDEX

This character was calculated by dividing the number of branches terminating in staminate inflorescences, excluding suckers, by the total number of leaves and multiplying by 100. It is thus the number of male branches expressed as a percentage of total leaves or internodes of the main culm.

In normal maize none of the branches above the suckers bear staminate flowers, although staminate tips and perfect flowered spikelets are common abnormalities. In teosinte all primary branches normally end in a staminate inflorescence. There is, then, no overlapping of either of the varieties or species with respect to this character.

The F_2 hybrid plants ranged from 0 to 71 with the mean at 37. The distribution (fig. 14) is practically symmetrical and clearly unimodal.

There are four significant correlations, all in the direction of coherences. They are with nodes without branches, nodes above, alicole index, and branch silking first. Except in the correlation with alicole index, a physiological explanation is suggested.

MALE SECONDARIES

As a measure of this character, all secondary branches on the third branch from the top of the plant that bore staminate spikelets were counted.

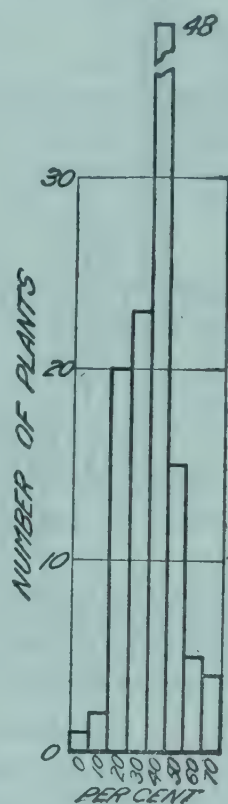


FIG. 14.—Male branch index: frequency distribution of plants in F_2 . Class value, 10 per cent.

In normal maize there would be no secondary branches bearing staminate spikelets. In Florida teosinte the number is usually 2 or 3. In the F_2 hybrid plants the mean number was 2. The range was from 0 to 8. Nearly half the plants had no staminate secondaries and there is almost no indication of a second mode (fig. 15).

Nearly all the significant correlations not readily assignable to physiological relations are disherent. Thus height, total leaves, and circumference index in the height group, secondary branches and secondary index in the tassel group, branch silking first, and days to pollen all show disherent correlations. Many of these are related, since they would be similarly affected by changes in vigor, but it is difficult to

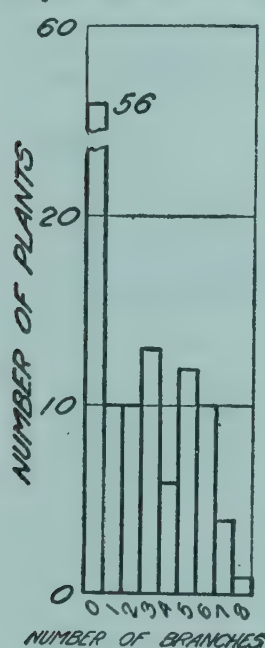


FIG. 15.—Male secondaries: frequency distribution of plants in F_2 . Class value, one branch.

understand why increased vigor should result in a smaller number of male secondaries; and the negative correlation with secondary index is difficult to understand as other than genetic.

The absence of correlation between male secondaries and male branch index, which are placed in the same group because both are measures of the tendency to produce staminate spikelets, is in itself an indication of disherence.

CHARACTERS OF THE PISTILLATE INFLORESCENCE

ALICOLE GROUP

To discuss the characters of the pistillate inflorescence of the hybrids between maize and teosinte, a short preliminary description is necessary.

In maize both staminate and pistillate spikelets are borne in pairs. In the pistillate inflorescence each pair of spikelets occupies a pit or alveolus. In the staminate inflorescences there is only a faint suggestion of an alveolus. In teosinte the arrangement of the staminate spikelets is like that in maize; but in the pistillate inflorescence the spikelets are borne singly, each occupying a highly specialized alveolus. In hybrids of maize and teosinte, all permutations of the above arrangements occur, and to facilitate description the term alicole is used for the spikelet or spikelets arising from a single alveolus or having a common origin. Thus an alicole may consist of one or more staminate spikelets, one or more pistillate spikelets, or both pistillate and staminate spikelets.¹

¹ For a more complete discussion of the pistillate inflorescence of teosinte and maize hybrids see COLLINS, G. N. STRUCTURE OF THE MAIZE EAR AS INDICATED IN *ZEA-EUCHLAENA* HYBRIDS. In *Jour. Agr. Research*, v. 17, no. 3, p. 127-135, 1 fig., pl. 16-18. 1919.

In normal maize the number of rows of alicoles is always half the number of rows of grains. In the hybrid plants, however, 4-rowed spikes may consist of either two rows of alicoles, each with two seeds, or four rows of alicoles, each with a single seed. Plants exhibiting the range of variation with respect to the pistillate inflorescence are shown in Plates 2 to 5.

As a basis of comparing the pistillate inflorescences of the hybrid plants, the best-developed spike on the third branch from the top of the plant was chosen and the number and nature of the alicoles were recorded. To eliminate as far as possible differences associated with the size of the spike, the number of alicoles of the classes single male, double male, single female, double female, and mixed (one male and one female) was expressed as a percentage of the total number of alicoles in the spike.

DOUBLE MALE ALICOLES

Neither maize nor teosinte normally produces male spikelets in the pistillate inflorescences. In the F_2 hybrids, however, out of 123 plants in which the nature of the pistillate inflorescences was determined, 18 had some alicoles with two staminate spikelets, in 2 plants the number being as high as 50 per cent (fig. 16).

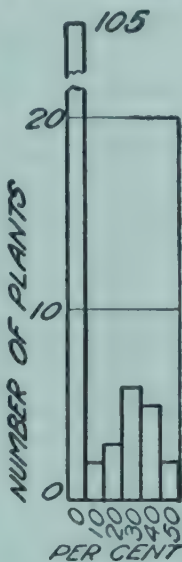


FIG. 16.—Double male alicoles: frequency distribution of plants in F_2 Class value, 10 per cent.

MIXED ALICOLES

Mixed alicoles are not a character of either maize or teosinte. There were, however, 13 F_2 hybrid plants with mixed alicoles, the highest percentage being 40 (fig. 17).

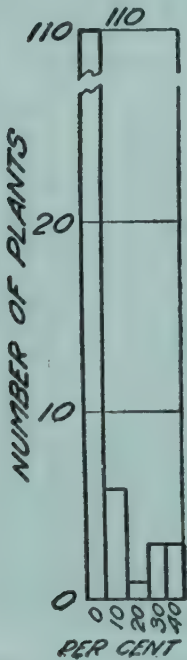


FIG. 17.—Mixed alicoles: frequency distribution of plants in F_2 Class value, 10 per cent.

SINGLE FEMALE ALICOLES

Single female alicoles are a universal character of teosinte, while in maize no variety is known in which the seeds are not paired. Single female alicoles may occur in rare instances on a part of an ear of maize where the number of rows is reduced toward the tip.

In the F_2 hybrid plants, although there was practically a continuous series from 0 to 100 per cent, there were distinct indications of a tendency to segregate into the two parental forms, there being two modes, one at 0, the other at 100 (fig. 18). The numbers at these two modes were 34 and 12, indicating that the maize character is dominant.

In this connection it should be recalled that in the F_1 plants the alicoles of the pistillate inflorescence all bore two spikelets.



FIG. 18.—Single female alicoles: frequency distribution of plants in F_2 . Class value, 10 per cent.

or the double alicoles of maize to predominate is the nearest approach to Mendelian behavior among the characters recorded.

The measurements of the alicole group form such a closely related series that their correlations may be discussed together. Significant coherences are shown with both characters of the male branch group and with number of alicoles, rows in the central spike, and number of suckers. The only significant disherence is between single female alicoles and primary branches.

Some of the coherences may be of a physiological nature, but the almost complete absence of any evidence of disherence with this group of characters which most nearly approaches an alternative method of inheritance should perhaps be noted.

DOUBLE FEMALE ALICOLES

Double female alicoles may be considered allelomorphic to single alicoles, but owing to the occurrence of plants with small percentages of double male and mixed alicoles the percentages are not exact reciprocals. There is, however, the same bimodality (fig. 19), the numbers indicating the dominant nature of this character.

ALICOLE INDEX

With the idea that mixed and male alicoles were in the nature of abnormalities, the number of single female alicoles was expressed as a percentage of the combined single and double female alicoles. There were 36 plants with no single female alicoles and 19 with no double female alicoles.

If the individuals are separated into two groups at the low point in the bimodal curve, which is 50 per cent, the numbers are 83 below this point and 37 above (fig. 20).

The tendency for either the single alicoles of teosinte

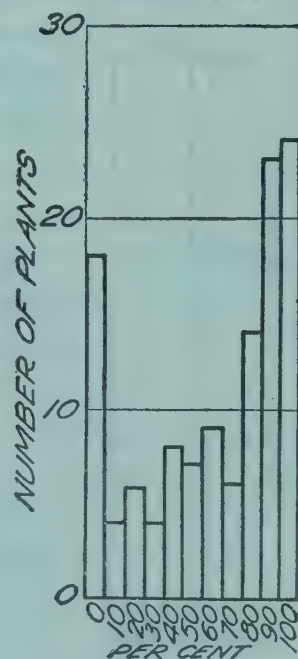


FIG. 19.—Double female alicoles: frequency distribution of plants in F_2 . Class value, 10 per cent.

NODES SILKING GROUP

NODES SILKING ON THIRD

This character, which is the number of nodes producing silk on the third branch from the top, was chosen with the idea of indicating the distinction between teosinte and maize with respect to the production of secondary fruiting branches on the upper part of the main culm. In all varieties of maize, branches from the upper part of the plant are normally simple, though secondary ears are a common abnormality. The branches of the ears of *Zea ramosa* are not subtended by bracts, and they arise from separate internodes only in the sense that branches from the tassel represent separate internodes.

Teosinte normally produces silks at two or three nodes of the third branch from the top. The average for 87 Florida teosinte plants was 2.3, with a range from 0 to 4. Since there are seldom more than 4 nodes on the third branch, the difference is more significant than the numbers would make it appear.

In the F_2 hybrid plants the range was from 0 to 10, with the mean at 5.5. The distribution (fig. 21) is practically symmetrical and unimodal.

There are three significant correlations with this character, but all appear to be physiological. The positive correlation with nodes on third branch is obviously almost physical; that with male secondaries is only slightly less so. The correlation with position of best spike of 0.47 might be considered a disherence, but it seems not unreasonable that with more nodes silking the best spike would, on the average, be located farther from the base. This is supported by the negative correlation of node silking index with position of best spike.



FIG. 21.—Nodes silking on third: frequency distribution of plants in F_2 . Class value, one node.

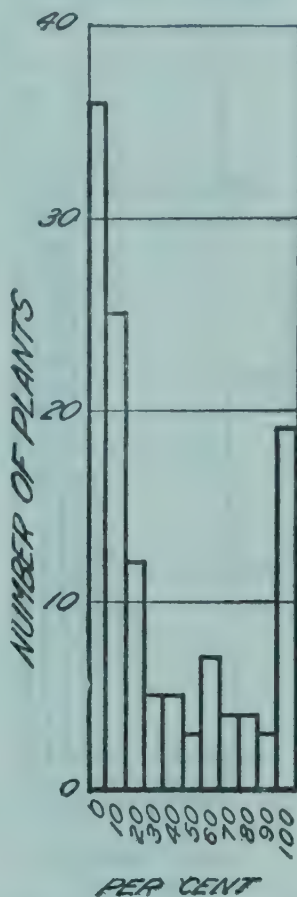


FIG. 20.—Alico Index: frequency distribution of plants in F_2 . Class value, 10 per cent.

NODES SILKING INDEX

The number of secondary branches silking as expressed in the preceding character is very definitely associated with the length of the third branch, the branches with more nodes having the greatest number

silking. With a view to obtaining an expression of the tendency to produce secondary branches independent of the length of the primary branch, the number of nodes silking on the third branch was expressed as a percentage of the total number of nodes on the branch.

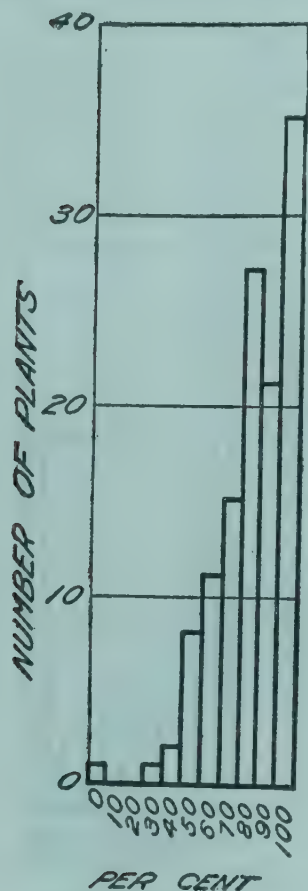


FIG. 22.—Nodes silking index: frequency distribution of plants in F_2 . Class value, 10 per cent.

character is therefore one that is sharply contrasted in the parents. Two of the F_1 plants in which this character was recorded each produced a single prophyllary spike.

In the second generation, 23 of the plants either had no prophyllary branch or it was not sufficiently developed to bear a spike. In 23 plants the branch consisted of an unbranched spike. The remaining 68 plants had from 2 to 14 spikes. The mean number for all plants was 3.1, the distribution (fig. 23) being skew but with no evidence of more than one mode. The three significant correlations are all coherent, but all may be physiological.

In teosinte the percentage is normally 100,¹ in maize 0. In F_2 hybrid plants the range was from 0 to 100. The modal number was 100, with the numbers diminishing with fair regularity to 0 (fig. 22). The mean was 78.

With the exception of the negative correlation with position of best spike, all the coherences are obviously physiological. On the other hand, the disherent correlation with height would appear to be genetic.

PROPHYLLARY GROUP

PROPHYLLARY SPIKES

Prophyllary branches are rare in maize and have never been observed in Tom Thumb. In varieties where prophyllary branches do occur they are simple. In teosinte, prophyllary branches are always well-developed; and in Florida teosinte, the average number of spikes is 6.3, with a range from 3 to 11. The disposition of the spikes in teosinte is shown in Table I. This

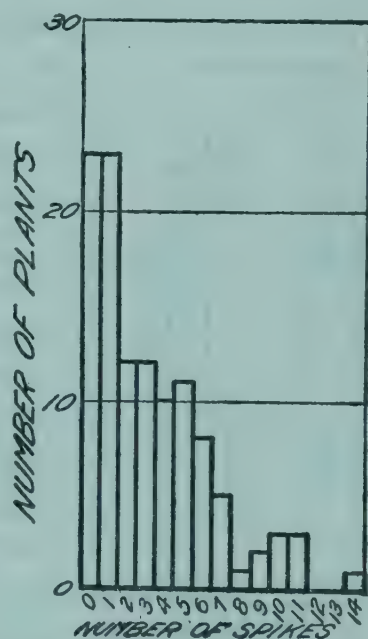


FIG. 23.—Prophyllary spikes: frequency distribution of plants in F_2 . Class value, one spike.

¹ This follows from the fact that although there is no branch produced in the axil of the uppermost leaf there is a fruiting branch borne in the axil of the prophyllum.

LENGTH OF PROPHYLLARY

This character is closely associated with the number of spikes in the prophyllary, and like that character it distinguishes sharply between the parental varieties and species.

The mean length of the prophyllary branch in 87 plants of Florida teosinte was 10.8 cm. The mean length in the F₂ hybrid plants was 13.4 cm. There was some evidence of two modes (fig. 24), one at 0, the other at 13.

There are three significant coherent correlations—namely, with male secondaries, nodes silking index, and length of internode on third.

The correlation with position of best spike is also significant but disherent.

Although prophyllary spikes and length of prophyllary have a positive correlation of 0.59, the first is negatively correlated with position of best spike while the correlation with the second is negative. Thus, as the prophyllary branch becomes longer there are more spikes, but they are smaller.

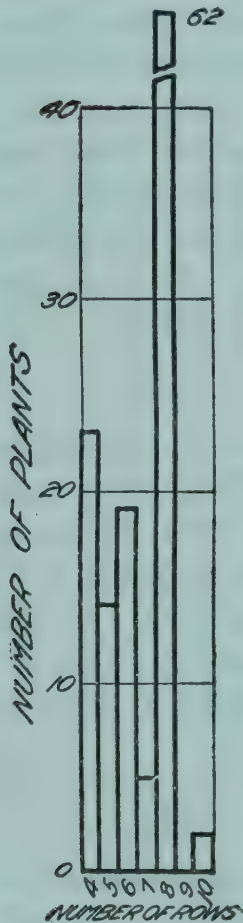


FIG. 25.—Rows in central spike: frequency distribution of plants in F₂. Class value, one row.



FIG. 24.—Length of prophyllary: frequency distribution of plants in F₂. Class value, 4 cm.

NUMBER OF ROWS GROUP
ROWS IN CENTRAL SPIKE

The number of rows of spikelets in the central spike of the tassel is a close homologue of the number of rows of seeds in the pistillate inflorescence. At first thought this might seem not to be the case in teosinte where all the spikes of the staminate inflorescence are 4-rowed and those of the pistillate inflorescence are 2-rowed. This apparent disagreement is occasioned by the suppression of one of each pair of spikelets in the pistillate inflorescence, there being in each instance 2 rows of alicoles.

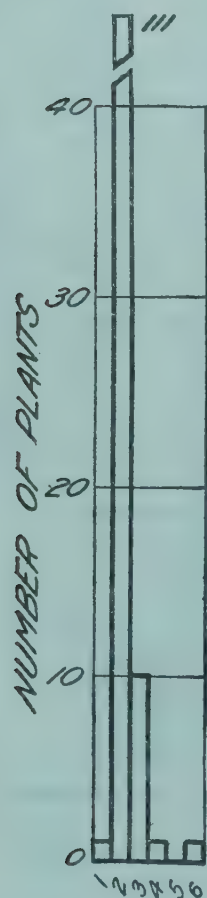
In maize, so far as observed, plants with 8-rowed ears always have 8-rowed central spikes. With the higher number of rows the arrangement in the central spike becomes indistinct.

In pure teosinte there is, properly speaking, no central spike, since the last division of the inflorescence gives two equal branches, each bearing 4 rows of spikelets. In the F_2 hybrid plants there were all stages from the condition found in teosinte, which was recorded as 4-rowed, to a well-formed central spike; and the number of rows is one of the best measures of the differentiation into a central spike. In many plants the number of rows was greater by 2 at the base of the spike than at the top. In such instances the intermediate odd number was assigned,

The distribution (fig. 25) was slightly bimodal, the modes being at 4 and 8, with the mean at 6.6.

All the significant correlations with this character are coherences, though none are very close.

It is of interest that the only other tassel character showing coherence with rows in central spike is secondary index. All other tassel measurements might be expected to increase with increased vigor; and since coherences would appear as negative correlations, any tendency for rows of central spike to increase with size would reduce the coherences. Two of the five alicole characters show significant coherences. There is also a significant coherence with number of alicoles.



NUMBER OF ROWS

FIG. 26.—Rows of alicoles: frequency distribution of plants in F_2 . Class value, one row.

ROWS OF ALICOLES

The number of rows of alicoles in the pistillate inflorescence is one of the most striking differences between teosinte and maize. In all varieties of teosinte the number is 2. The lowest number in maize is 4, as is characteristic of all 8-rowed varieties. In the large-eared varieties the number reaches 18. All the F_1 plants had uniformly 2 rows of alicoles, indicating the dominance of the teosinte character.

In the second generation 111 out of 123 plants also had 2 rows (fig. 26). This number is 19 in excess of the number expected if the character were behaving as a simple Mendelian unit. The uniformity of the F_2 plants with respect to this character made it impossible to determine correlations, but of the 12 plants with more than 2 rows of alicoles all but 1 had more than 4 rows in the central spike.

INDEPENDENT CHARACTERS

POSITION OF BEST SPIKE

In maize the pistillate spike is terminal on the branch. In teosinte there are usually a number of spikes of nearly equal size, the prophyllary branch usually producing spikes as large as any on the branch.

In the F_2 hybrid plants this character was determined on the third branch. The nodes were numbered from the base of the branch, the prophyllary branch being recorded as 0. The range was from 0 to 9, with the mode at 3. The mean was 2.22. The distribution (fig. 27) was decidedly

skew, but there was little evidence of more than one mode.

In its relation to other characters, this character is very irregular. The large number of disherent correlations may indicate that the terminal position of the pistillate inflorescence in maize is not inherited as a tendency for the lateral pistillate inflorescences to be located near the top of the branch. When secondary ears are developed in maize they are always near the base of the branch, and the expression of this tendency in inheritance may be the explanation of the apparently disherent correlations.

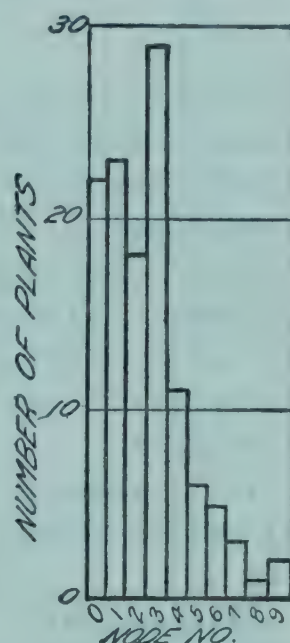


FIG. 27.—Position of best spike: frequency distribution of plants in F_2 . Class value, one node.

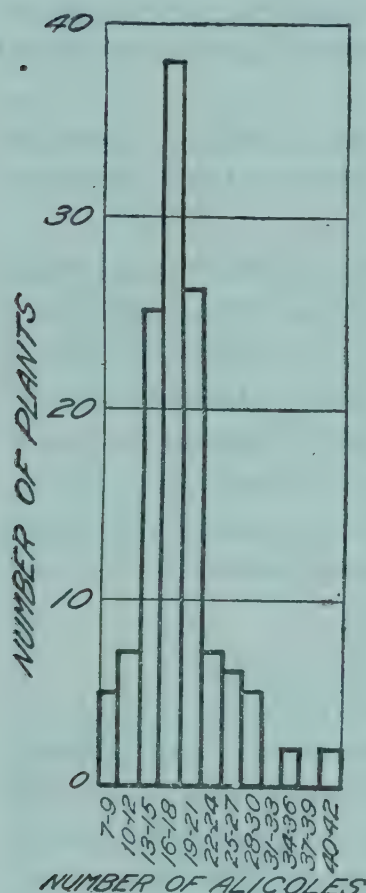


FIG. 28.—Number of alicoles: frequency distribution of plants in F_2 . Class value, three alicoles.

NUMBER OF ALICOLES

The number of alicoles in a well-developed spike of Florida teosinte is 7. In Tom Thumb maize the number is seldom less than 100. The lowest number recorded in a normal ear of maize is 50, in a Peruvian variety from the region of Lake Titicaca. This is, therefore,

one of the characters in which there is no approach to overlapping in the parental species.

The F_1 plants had spikes with from 11 to 18 alicoles. In the second generation the range was from 7 to 40. The mean was 17.85 with nearly symmetrical distribution (fig. 28), the mode being at 16.

The significant coherences with characters in the alicole group afford perhaps the most direct evidence that has appeared that the characters of the pistillate inflorescence tend to be inherited as a unit.

The correlation with rows in the central spike is perhaps physiological. There are no significant disherences.

NUMBER OF SUCKERS

Florida teosinte is characterized by a large number of suckers or branches that arise from below or near the ground. In a population of Florida teosinte at Chula Vista, grown in 1917, the average number of suckers was 14. Tom Thumb never produces suckers on normal plants, and no variety of maize has been studied that produces as many suckers as teosinte. The expression of this character is so dependent on environmental conditions, however, that statements regarding the range in maize would have little value. The most vigorous F_1 plant produced 11 suckers.

In the second generation the range was from 0 to 32, with the mode at 13 and the mean at 11.7. There is no evidence of more than one mode (fig. 29).

There are, in all, three significant correlations with this character, nodes without branches, single female alicoles, and double female alicoles—all of them coherences. The first of these is obviously physiological, since a large number of suckers and a small number of vacant nodes are both expressions of a tendency to produce branches. The other two are practically different expressions of the same character and indicate a coherence.

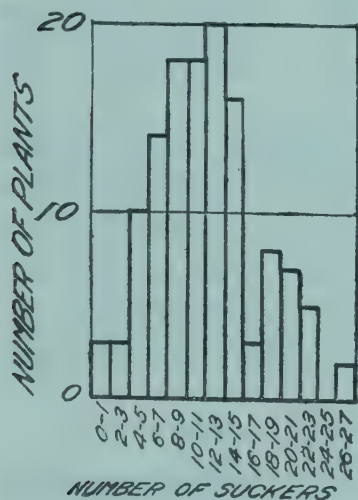


FIG. 29.—Number of suckers: frequency distribution of plants in F_2 . Class value, two suckers. One plant at 32.

BRANCH SILKING FIRST

In recording this character the primary branches were counted from the top. In maize the uppermost branch is the first to silk, except in rare instances where the second ear may silk a day or two in advance of the first. In teosinte the fourth or fifth branch is usually the first to silk. This character therefore distinguishes sharply between the parents with respect to both the variety and the species.

The F_2 hybrid plants ranged from 1 to 5, with equal numbers at 1 and 2. The mean was 1.9, the distribution (fig. 30) was skew and unimodal.

With the height group there are two significant correlations, one a coherence with total leaves, the other a disherence with sucker index. This disherence doubtless results from the negative correlation between total leaves and sucker index. The partial correlation of node silking first

with either total leaves or sucker index, with the other character constant, is less than three times the probable error. There are also significant correlations with all the characters of the nodes above group. These correlations are in a sense physical, since the value representing the node silking first must always be greater than the nodes above. In the male branch group there is a significant coherence with male branch index and a disherence with male branches on third. In addition there are significant coherences with secondary branches, position of best spike, and days to pollen.

DAYS TO POLLEN

Although profoundly influenced by the environment, the length of time before pollen is shed is the best measure of the length of season required for development. Under similar conditions there are few varieties of maize that require so long a time to mature as Florida teosinte, and Tom Thumb is one of the earliest varieties of maize. The period for Florida teosinte under conditions similar to those where the hybrid plants were grown was 162 days, and for Tom Thumb 43 days.

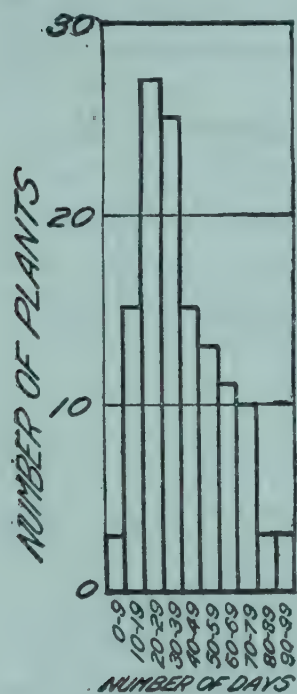


FIG. 31.—Days to pollen: frequency distribution of plants in F_2 . Class value, 10 days.

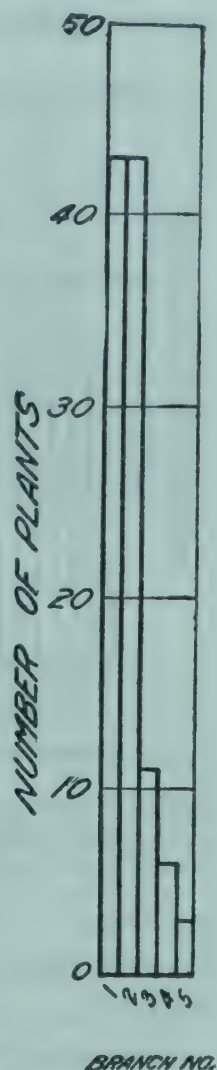


FIG. 30.—Branch silking first: frequency distribution of plants in F_2 . Class value, one branch.

The average time for the F_1 was 98 days. The F_2 plants averaged 112 days, with a single mode at 96 days (fig. 31). The earliest plant flowered in 71 days, and the latest required 165 days from the date of planting.

With characters of the height group there are two significant coherences, height and total leaves, and two significant disherences, sucker index and nodes without branches. The correlation with height is an especially striking coherence, since the positive correlation is 0.47 while the same correlations in both teosinte and Tom Thumb are negative, being 0.46 and 0.11, respectively. Days to pollen and total leaves in teosinte have a correlation of 0.14, a correlation significantly lower than the 0.79 of the hybrids.

The negative correlation with sucker index appears to result from the negative correlation of sucker index with total leaves.

The coherence with nodes above on third is barely significant and may be physiological. There are significant coherences with three of the four tassel measurements, and in Tom Thumb the three tassel measurements recorded are all negatively correlated with days to pollen.

There are also significant coherences with number of alicoles and node silking first. The disherent correlations with male secondaries and length of internode on third appear to be genetic. That with length of internode on third is the highest coefficient with days to pollen.

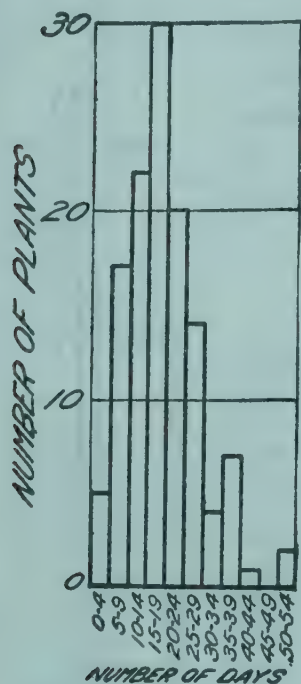


FIG. 32.—Pollen to silk; frequency distribution of plants in F_2 . Class value, five days.

POLLEN TO SILK

Maize is normally proterandrous. There are, however, proterogynous strains of maize, and proterogynous individuals in almost any strain are not uncommon. Tom Thumb is normally proterandrous by about 10 days. Florida teosinte appears to be normally proterogynous. It has always been so in our experiments; and an examination of the fields at Clarcona, Fla., in 1914, showed the plants to be silking from 7 to 10 days before pollen. Durango teosinte, on the other hand, under most conditions is proterandrous.

In both maize and teosinte this character is especially susceptible to environmental influence. The F_1 plants were decidedly proterandrous at both Lanham and Chula Vista. None of the F_2 plants were proterogynous, the proterandry rang-

ing from 0 to 53 days, with the mean at 18.3. The distribution (fig. 32) was symmetrical and unimodal.

There are but two significant correlations with this character, both coherences. These are with circumference index and position of best spike. The latter is in one sense physiological.

LENGTH OF INTERNODE ON THIRD

This character was determined by dividing the length of the third branch by the number of internodes. The branches from the upper nodes of a maize plant are much shortened. An accurate measure is impossible on account of the difficulty of accurately determining the number of nodes. In Tom Thumb it would, however, be somewhat less than 1 cm., and in normal maize plants of any variety it would scarcely exceed 3 cm. In a normally developed teosinte plant the internodes of the third branch will average about 10 cm. This character was not recorded in the first generation. In the F_2 plants the mean was 10.9 cm. The range was from 2 to 22, with practically a normal distribution (fig. 33).

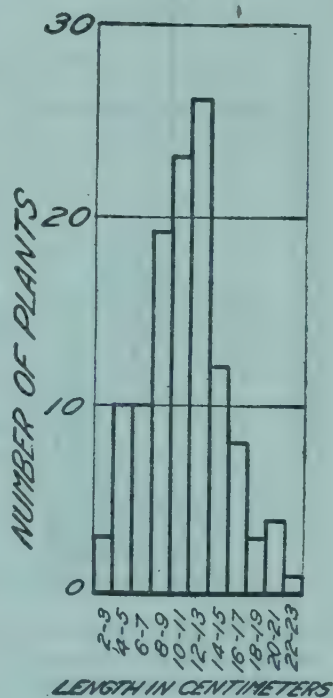


FIG. 33.—Length of internode on third; frequency distribution of plants in F_2 . Class value, 2 cm.

This character might be expected to be closely related to number of nodes on third, since in a mathematical sense it is a function of that character. However, the correlation between length of internode on the third branch and nodes on third is -0.05 .

Length of internode on third shows a larger number of significant correlations than does any other character.

A high expression of the character might be expected to be associated with increased general vigor, but many of the correlations are in the opposite direction. There is distinctly more evidence of disherence than of coherence. In fact, three of the four significant coherences may be physiological, while most of the disherences are not to be explained in this way.

Especially significant are the pronounced negative correlations with height and total leaves. Only slightly less striking are the negative correlations with two of the tassel characters.

DISCUSSION OF CORRELATIONS

It would be very difficult, if not impossible, to determine with accuracy the number of independent correlations. The interrelation of the characters is of a most intricate nature; and even if the data warranted the calculation of the partial correlations of each pair with all other characters constant, the facts would still be very inadequately represented. Correlations take no account of causation or the sequence in which characters are determined.

It is clear that the values of some characters are directly influenced by others, the relation being causal in nature. Thus the number of total leaves acts as a limiting factor to the number of branches ending in male flowers, and the correlation of any character with number of branches ending in male flowers may to some extent follow as a secondary relation to the correlation between the character in question and total leaves. On the other hand, it is obviously absurd to reason that branches ending in male flowers may influence the total leaves; and to correct the correlation with total leaves by making branches ending in male flowers constant might represent a mathematical relation, but the determination would have no biological significance.

An attempt was made to determine whether the more striking disherent correlations might result from the correlations of other interrelated characters. Thus height and the index of nodes silking on the third branch, which showed a disherent correlation of -0.46 , were found to be mutually correlated with four other characters to an extent that would materially influence the correlation in question. The partial correlation of height and index of nodes silking on the third branch with all of the

four correlated characters constant was found to be -0.69 . Such relations must stand, therefore, as disherences so far as the recorded data are concerned.

A study of the correlations shows that within wide physiological limits there are no incompatible combinations. On the other hand, all the characters are in a sense interrelated. Having in mind the theory that ascribes the determinants of characters to definite locations on the chromosomes, the author's examined the correlations to determine whether there were groups of characters between which there were no significant correlations. No such grouping was apparent, and it was possible to arrange the entire series so that they formed a single group with no correlation lower than ± 0.31 .

If the results of this experiment are interpreted in terms of the theory mentioned above, it follows from the blended character of the inheritance that practically all the characters result from the combined action of numerous factors. The failure of the characters to fall into groups the members of which are genetically correlated further indicates that the factors for the individual characters must be distributed in different chromosomes.

CORRELATION AMONG DESIRABLE CHARACTERS

Among the characters measured, a certain few are indicative of desirable characteristics from the standpoint of a forage plant. The more important of those are (1) total leaves, indicative of the luxuriant foliage of the teosinte, (2) circumference index, a small circumference in proportion to the height indicating the slender, edible stalks of the teosinte, (3) nodes silking on third branch, indicating the profuse production of seed of the teosinte, (4) number of suckers, indicating the abundant production of forage of the teosinte, (5) male branch index, indicative of the numerous branches of teosinte, (6) number of alicoles in the best spike, indicating the large pistillate inflorescences of maize, (7) rows in the central spike, indicating the many-rowed inflorescences of maize, and (8) days to pollen, a low value indicating the short season of maize.

The interrelation of these selected characters is shown in Table VI. Of the 27 combinations of these characters there are 9 in which both of the desired characters are possessed by teosinte, 3 in which both are possessed by maize, and 15 where it is desired to combine teosinte and maize characters.

TABLE VI.—Correlation of characters desirable in a forage plant^a

Characters considered.	Circumference index.	Nodes silking on third.	Number of suckers.	Male branch index.	Number of alicoles.	Rows in central spike.	Days to pollen.
Total leaves.....	-0.31	0.05	-0.10	-0.07	-0.18	-0.14	0.79
Circumference index.....		.00	-.19		.12	-.03	-.17
Nodes silking on third.....			.01	-.19	.05	-.03	.07
Number of suckers.....				.14	-.11	.02	-.09
Male branch index.....					.06	-.04	.03
Number of alicoles.....						.37	-.29
Rows in central spike.....							-.09

^a Figures in bold-face type indicate coefficients of correlation between the characters where a combination of teosinte and maize characteristics is desired.

Of the 15 character pairs where new combinations are desired, there is only one significant correlation. This is days to pollen and total leaves. In this one instance the relation is in a sense physical, since there is obviously a physical limit to the number of leaves that can be developed in a very short season. The indications from this comparison are, therefore, that coherence presents few obstacles to the securing of desired combinations. (Pl. 2; 6, A, B.)

Another view of the comparative independence of the characters may be gained by an examination of the plants that were most like maize or teosinte with respect to some of the more important characters. Table VII is provided to make this possible. Each pair of columns gives the measurements for two plants, one of which was the most like maize and the other the most like teosinte with respect to the character named at the head of the column.

TABLE VII.—Comparison of individual plants, showing the extreme variations toward maize and teosinte, respectively^a

Characters considered.	Average.	Height.		Total leaves.		Height of sucker.		Sucker index.		Circumference index.		Male branches. ^b		Number of suckers.		Number of alicoles.		Days to pollen.		Rows of alicoles.																		
		Maize-like (plant 19).		Teosinte-like (plant 38).		Maize-like (plant 78).		Teosinte-like (plant 20).		Maize-like (plant 78).		Teosinte-like (plant 19).		Maize-like (plant 115).		Teosinte-like (plant 64).		Maize-like (plant 46).		Teosinte-like (plant 62).		Maize-like (plant 81).		Teosinte-like (plant 35).		Maize-like (plant 136).		Teosinte-like (plant 24).		Maize-like (plant 27).		Teosinte-like (plant 38).		Maize-like (plant 36).		Teosinte-like (plant 49).		
Height.....	14	4	23	14	22	14	20	14	4	8	17	...	22	9	14	15	17	9	23	14	11																	
Total leaves.....	23	22	33	13	38	23	26	23	22	...	23	29	38	19	20	23	28	21	33	19	24																	
Height of sucker.....	16	20	21	17	23	6	27	6	20	18	17	7	23	0	16	16	19	13	21	15	12																	
Sucker index.....	112	460	90	130	100	50	140	50	460	230	100	...	100	0	110	110	110	140	90	110	110																	
Circumference index.....	4.5	...	3.5	...	4.4	4.0	...	4.0	...	9.1	2.4	...	4.4	3.5	4.3	5.5	...	5.0	3.5	4.0	5.0																	
Male branches ^b	8	9	5	12	21	5	5	5	9	18	9	0	21	5	6	10	9	6	5	5	7																	
Number of suckers.....	12	14	8	21	7	4	15	4	14	11	14	2	7	0	23	18	20	15	8	3	15																	
Number of alicoles.....	18	19	17	17	34	21	18	21	19	23	21	29	34	17	9	40	7	12	17	30	9																	
Days to pollen.....	112	103	165	103	121	100	108	100	103	142	106	...	121	129	138	100	131	69	165	85	116																	
Rows of alicoles.....	2.1	2	2-3	2	3	2	2	2	2	3	2	2-4	3	2-3	2	4	2	2	2-3	6	2																	

^a Each pair of columns gives the measurements of two plants, one of which was most like maize and the other most like teosinte with respect to the character given at the head of the columns. The value of the character for which the plant was selected is given in bold-face type. For description of units of measurement, see p. 7-8.

^b The number of primary branches that terminate in a staminate panicle, exclusive of suckers.

It may seem that, except for the character chosen, the values for the most part depart little from the mean values. For example, under total leaves the most maize-like plant which had 13 leaves was particularly maize-like in no other character. It was even below the average in number of alicoles in the best spike and had almost the maximum number of suckers. On the other hand, the plant with the greatest number of leaves had also the greatest number of male branches but was decidedly maize-like with respect to number of suckers and number of alicoles.

CONCLUSIONS

The genetic relations of the principal characters of maize and teosinte were investigated in a cross between a small variety of pop corn and Florida teosinte, a large forage grass generically distinct from maize. The F_1 plants showed characters which, for the most part, were intermediate between those of the parents.

The F_2 plants were also intermediate, with a greatly extended range of variation. Thirty-three of the characters that differentiate the parents were chosen and recorded for each of the 127 F_2 plants. The distribution of these characters with one or two exceptions showed little or no evidence of alternative or Mendelian inheritance.

With respect to the individual characters, the extreme variants approached, and in some instance exceeded, those of the parents; but none of the plants possessed any large number of the characters of either maize or teosinte.

The results showed the greatest freedom of recombination. All combinations of characters appeared that might reasonably be expected with so limited a number of individuals. There were many instances of coherence or partial coupling, but there was an almost equal number of instances where characters derived from different parents showed a tendency to combine more frequently than would be expected as the result of chance. In such a complicated series it was found impossible, however, to distinguish primary from secondary correlations.

While there appeared to be no incompatible combinations, there were, on the other hand, no completely independent characters. Every character recorded showed significant correlation with one or more other characters; and these in turn were correlated with still others, with the result that all the characters were interrelated and formed a single group. It is possible, in fact, to arrange all the characters in such a way that they form a single group in which there is no coefficient of correlation lower than ± 0.31 .

The nearest approach to Mendelian inheritance was shown by the arrangement of the spikelets in the pistillate inflorescence (fig. 18, 19, 20). In maize the female spikelets are borne in pairs (double female alicoles); in teosinte the female spikelets are borne singly (single female

alicoles). Dominance of the maize character was complete in the first generation. In the second generation the segregation was not complete, there being many plants with both single and double female alicoles; but the number of individuals in which double female alicoles predominated was approximately three times the number in which there were more single female alicoles.

It was found that the characters of the pistillate inflorescence were subdivided in transmission to a remarkable degree. Thus the maize ear, instead of behaving as a unit, was subdivided into a large number of separately inherited units, such as number of rows, closely crowded seeds, and shortened peduncles, all of which were inherited more or less independently. Number of rows was still further resolved into paired or single spikelets and the number of rows of alicoles in which they were borne.

A surprisingly large number of the plants combined the abundant production of suckers characteristic of the teosinte parent with the sturdy, upright character of maize and resulted in very leafy, compact plants of a type that should prove valuable for forage purposes. (See Pl. 6, A.)

It remains to be seen whether the new combinations can be maintained and made to breed true. The results of previous experiments with maize hybrids would indicate that selection for a few generations will fix any desired combination.

PLATE I

A.—General view of F_2 plants of teosinte-maize hybrid.

B.— F_2 plants of teosinte-maize hybrid, showing diversity in size and season.



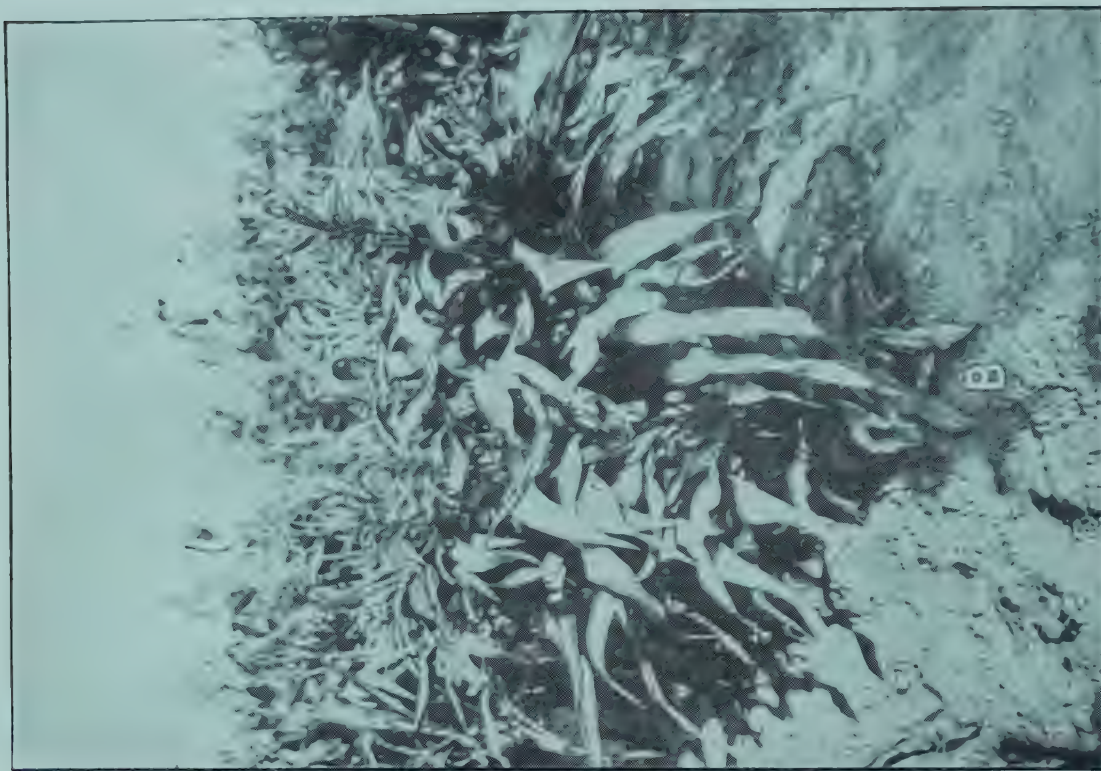


PLATE 2

Teosinte-maize hybrid:

A.—F₂ plant No. 36. This plant bore the most maize-like pistillate inflorescence that appeared in the second generation.

B.—F₂ plant No. 49. The pistillate inflorescences of this plant were among those most nearly resembling teosinte.

PLATE 3

Teosinte-maize hybrid:

Pistillate inflorescence of F_2 plant No. 36, shown in Plate 2, A. Natural size.

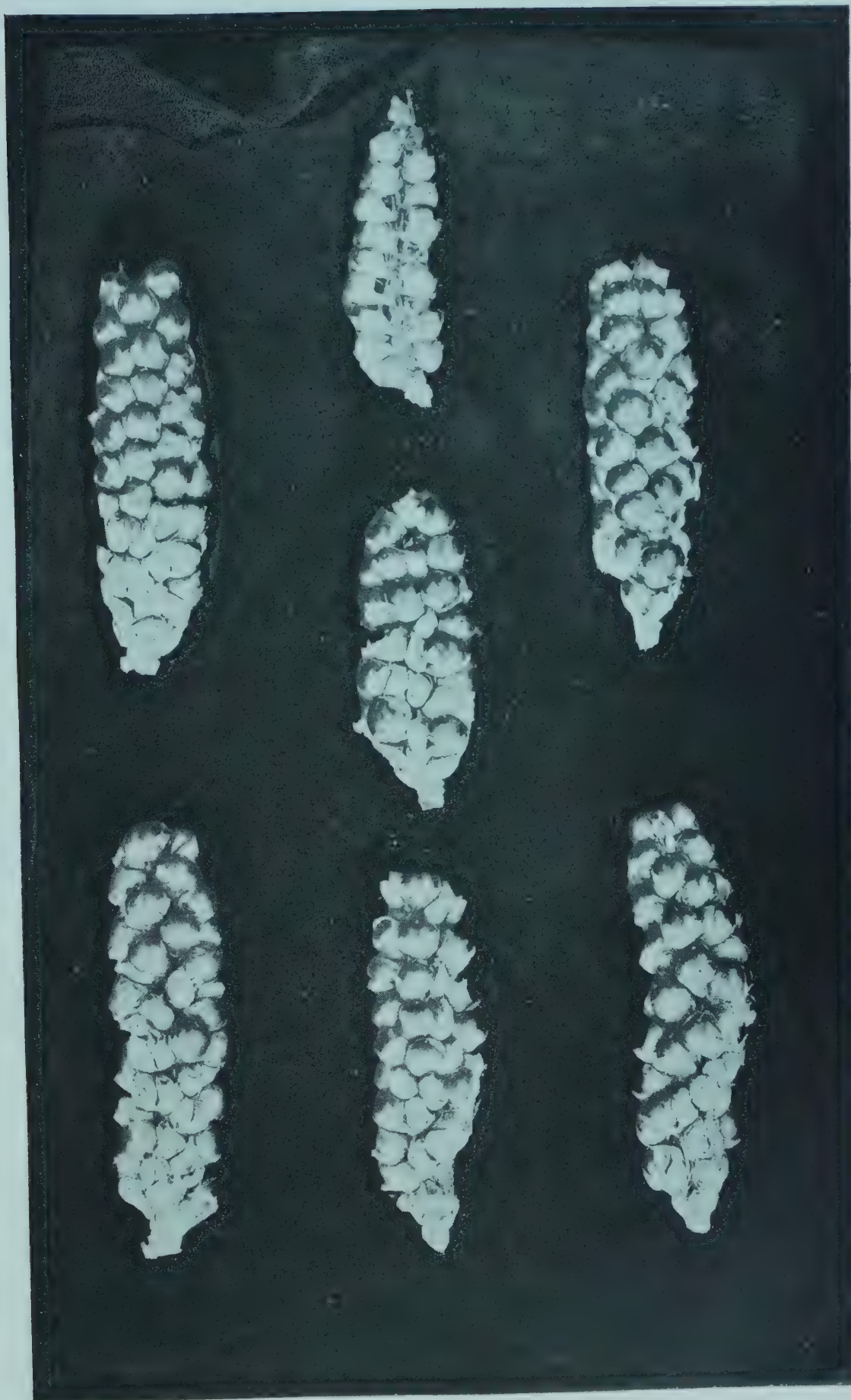


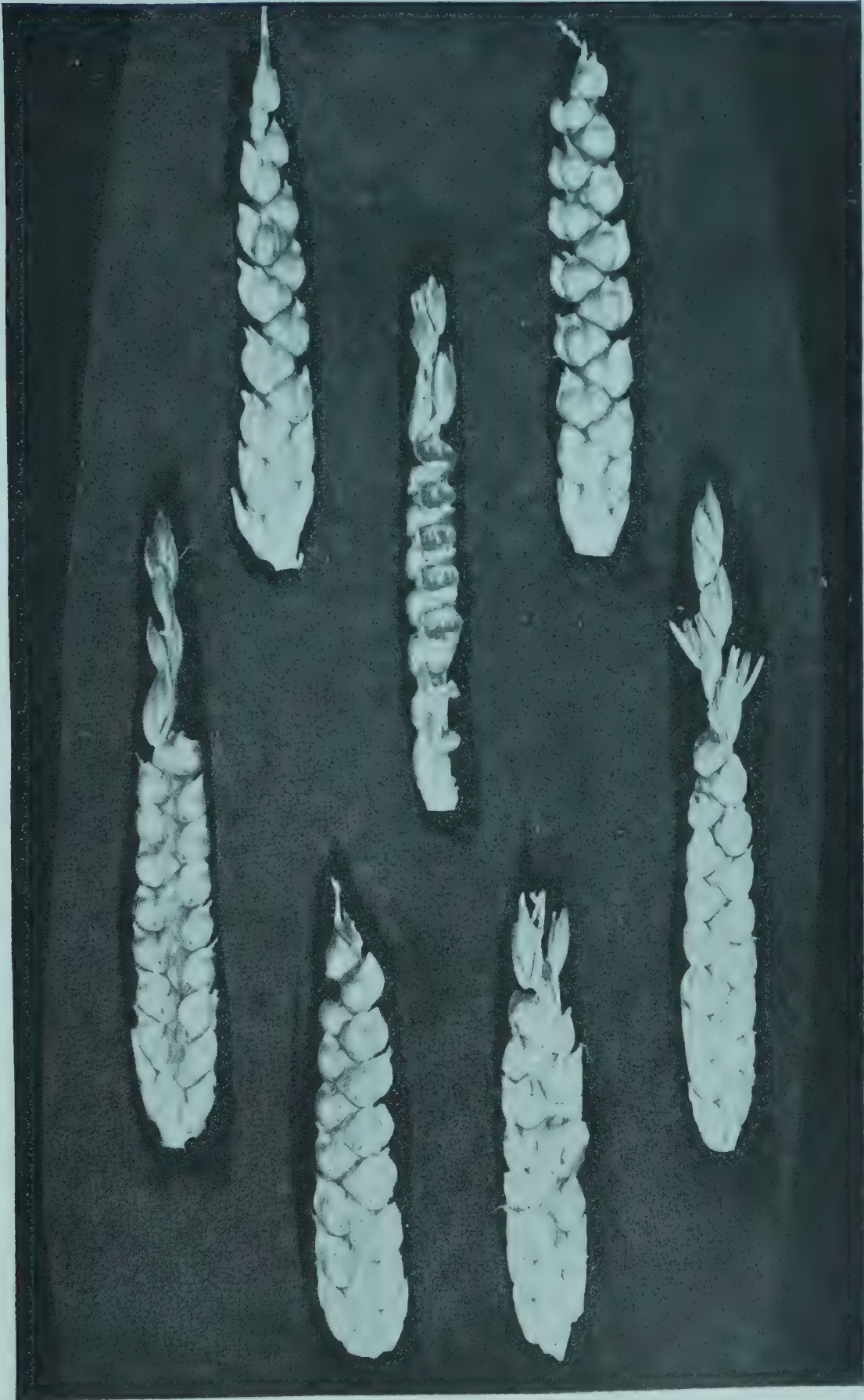


PLATE 4

Pistillate inflorescence of plant No. 49, shown in Plate 2, B.

PLATE 5

Pistillate inflorescences from plant No. 94, illustrating an intermediate type of inflorescence. The arrangement of the alicoles is much like that of teosinte, but 90 per cent of the alicoles are double female.



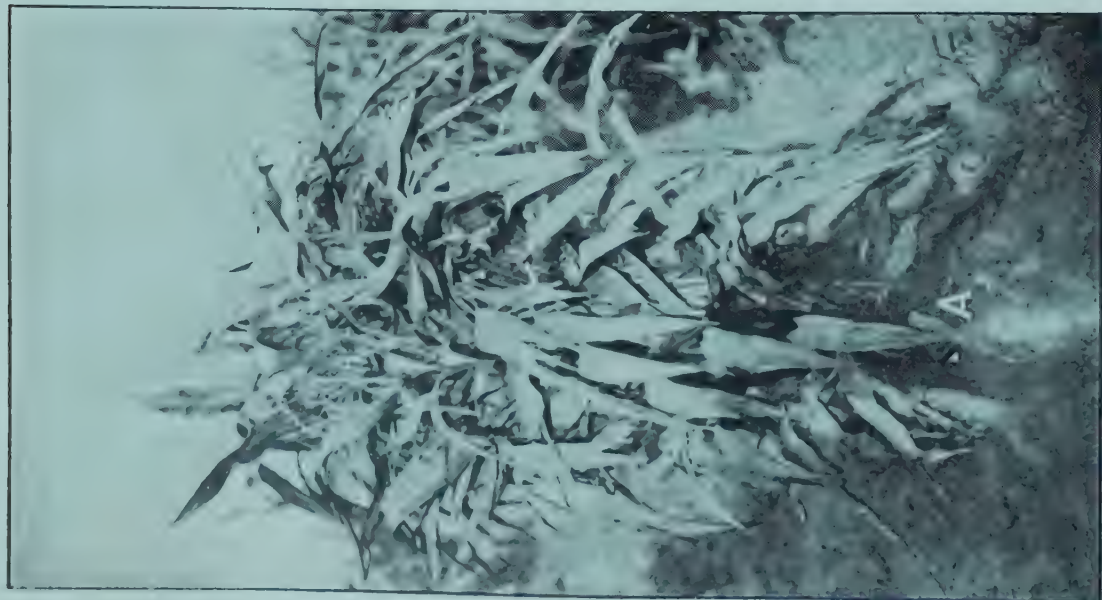
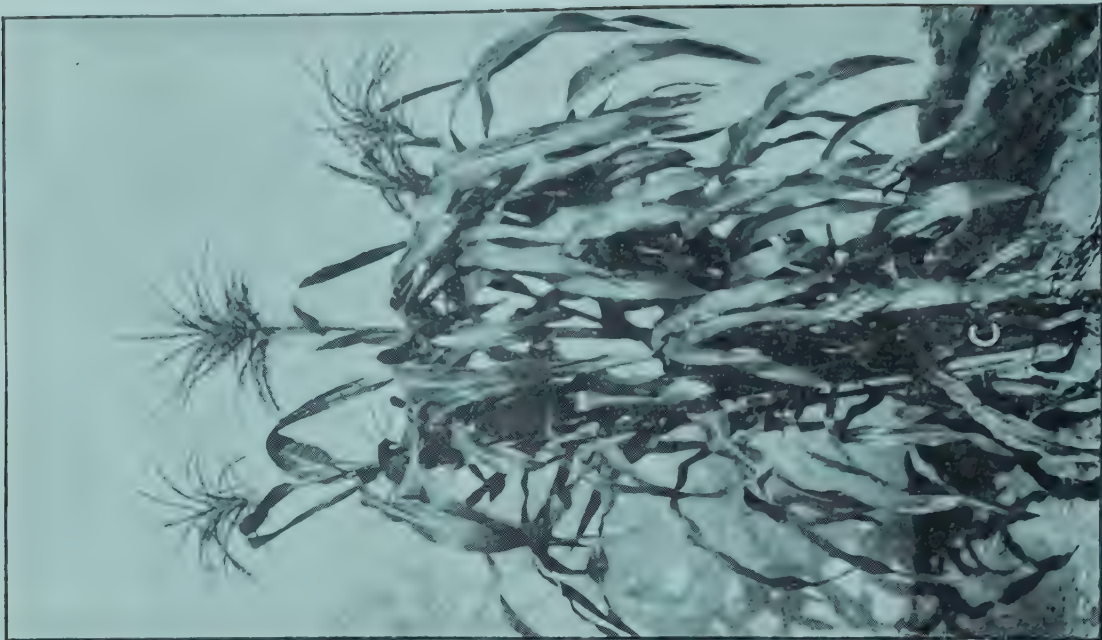


PLATE 6

Teosinte-maize hybrid:

A.—F₂ plant No. 31, showing compact growth characteristic of many of the plants. Although only 14 dcm. high, this plant had 30 leaves on the main culm, nearly equaling teosinte in this respect. The plant resembled maize in having no spikes developed in the axil of the prophyllum.

B.—F₂ plant No. 113, showing stiff, erect leaves. This plant resembled teosinte in being very late in maturing, yet it was among the most maize-like with respect to circumference index.

C.—F₁ plant, grown at Lanham, Md.

PLATE 7

Teosinte-maize hybrid:

Pistillate inflorescences of the F_1 plant shown in Plant 6, C.



BANANA ROOT-BORER

By G. F. MOZNETTE¹

Entomological Inspector, Tropical and Subtropical Fruit Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

The existence in Florida of a root-weevil peculiar to the banana was brought to the writer's attention in December, 1917, by the receipt of some specimens from a grower near Larkins, in Dade County, Fla., who advised the writer of serious damage to his banana plants. The insect was determined by Dr. W. Dwight Pierce at Washington, D. C., as the banana root-borer, *Cosmopolites sordidus* Germar, a dangerous banana pest prevalent in almost every section where bananas are grown for commercial purposes. Since this species and all plants infested with it had been declared to be public nuisances in Florida, the State Plant Board at Gainesville, Fla., was immediately notified, and eradication and inspection work was begun. It was during the eradication and inspection work that the writer, cooperating with members of the State Plant Board, was enabled to make a number of observations on the habits of this species; and it was thought well to publish the following data to aid others who may find this pest of the banana in the State of Florida or wherever bananas are grown.

A national quarantine was placed on this species April 1, 1918. This quarantine forbids the importation into the United States from foreign countries where the banana root-borer exists of all species and varieties of banana plants (*Musa* spp.) or portions thereof, except for experimental and scientific purposes.

The spread of the insect from one country to another is probably accomplished by the transportation of infested suckers for planting (11, p. 33-34);² and its spread within any locality most likely follows the killing out of infested stools, after which the adults travel in search of fresh supplies of food plants. Within a locality they could also be spread by the transportation of infested suckers or young plants for propagation.

HISTORY AND DISTRIBUTION

The adult (Pl. 8) was described as *Calandra sordida* by Germar (6) in 1824. The genus *Cosmopolites* was established for this species by Chevrolat (3) in 1885.

E. Fleutiaux (5) recorded it from Madagascar in 1903, stating that it was a serious enemy of the banana on that island. In 1908 C. H. Knowles (9) mentioned carbon disulphid as a means of control in the

¹ Technical descriptions of the stages of the weevil by W. Dwight Pierce.

² Reference is made by number (italic) to "Literature cited," p. 46.

Fiji Islands. In 1912 H. A. Ballou (1, p. 112) reported the species as doing serious damage to bananas in the Lesser Antilles.

During 1914 T. Fletcher (4, p. 342-343, fig. 201) published records of this species from southern India as existing in the regions of Malabar, Caimbatore, Godavari, and Ganjam. In the same year Frank P. Jepson (8), then working with the species in the Fiji Islands, where it is serious, made a mission to Java in quest of the natural enemies of the species and brought into the Fiji Islands some predatory beetles. He was successful in introducing some histerid beetles which were keeping the borers down in Java.

Later in 1916 Ballou (2) reported this insect as widely distributed in the Tropics, it being found in Jamaica, Guadeloupe, Dominica, Martinique, and Trinidad in the West Indies; Brazil in South America; and the Philippines, Fiji, Borneo, Sumatra, India, Queensland, and the Straits Settlements in the East.

Besides the localities cited, Frank P. Jepson (8) in 1914 recorded additional places where it is found: Java, Ceylon, New Guinea, Malacca, Saigon, China, Raratonga, Reunion, Sikhim, North Bengal, Pequ, Tenasserim, Andaman Islands, Sambak, and the Seychelles.

In Florida investigations showed that the infested plantings at Larkins had all been made four years previous to the discovery of the weevils, with plants procured from a nursery in the northern part of Florida which had, in turn, secured the plants from a nursery in southern Florida. In March, 1918, the weevils were found at the nursery in southern Florida, and every effort was made to exterminate them. It may be that many shipments of infested plants were made from this source, and it is very important that every occurrence of this pest be located and eradicated. Since the insect attacks sugar cane also it is not improbable that its presence would seriously interfere in the future with the development of large sugar and sirup industries. It is not known how this insect found its way into Florida, but no doubt it came in with sprouts or young plants introduced for propagation.

HOST PLANTS

According to published records there does not seem to be a great variety of host plants, *Cosmopolites sordidus* apparently having confined itself thus far almost entirely to the banana, attacking all varieties. The borer has been reported, however, as attacking sugar cane. In Fiji, Jepson (7) states that the borer does not appear to display more partiality for one variety of banana than another.

CHARACTER OF THE INJURY

The young suckers attacked by the borers wither and die in a very short space of time. This is due to the feeding and tunneling of the grubs or larvæ between the lateral roots and the bulb (Pl. 11, B), thus cutting off the flow of sap to the plant. The banana plant has no central

tap root, but is supported by numerous lateral roots (Pl. 11, A). An indication that a young plant is infested is the withering and drying of the curled roll of unopened leaves or growing part of the plant. The root, upon examination, is found to be riddled with the larvæ of this insect and when cut open discloses the borer *in situ*. The adult weevils are abundant in the soil about the root and also are found under loose fiber surrounding the base of the stem, at the crown. They also congregate in the cavities caused by the larvæ at the base of the bulb of the banana plant. In the planting at Larkins, Fla., where the infestation was first found, the writer collected 55 adults at the base of one plant and as many as 60 larvæ and pupæ in the bulb. The older plants infested appeared tall and spindling and no doubt succeeded in growing as much as they did by the presence of numerous lateral roots surrounding the bulbs of the plants and because the attacks of the insects had been gradual. Most of the bananas in the planting were old and so riddled by the larvæ as to be readily felled. After feeding thoroughly on a plant the weevils abandon it for another.

TECHNICAL DESCRIPTIONS OF THE SPECIES

The following descriptions by Dr. W. Dwight Pierce are based upon specimens collected at Larkins, Fla., January 19, 1918. The fine drawings accompanying the descriptions were made under Dr. Pierce's supervision by Mr. Harry Bradford and by Dr. Adam Böving.

EGG

The egg is elongate oval, about 2 mm. in length, rounded at one end and more or less pointed at the other, and pure white in color.

LARVA (Pl. 9, B-G)

The larva is characteristically calandrid in form (Pl. 9, B), having the eighth and ninth segments transformed into a sort of pygidial plate bearing very large elongated spiracles on the eighth segment (Pl. 9, F, G). The other abdominal spiracles are all very minute and indistinct. The mesothoracic spiracles are very large. The length of a full-grown larva is at least 13 mm. (The writer has not had a live specimen to measure.) The body is white and the head shield dark reddish brown. The head is quite prominent. The head shield is broadly, elongately emarginate behind (Pl. 9, C). From the center of the emargination on the median line the epicranial suture passes forward, separating the epicranium into two parts (Pl. 9, C). This suture is strongly marked with black on its posterior half and is white from thence forward to the frons, behind which it divides and forms two frontal sutures (Pl. 9, D).

The frons (Pl. 9, D) is subtriangular, emarginate at anterior angles for the antennæ, and emarginate along the epistoma for attachment of the clypeus. The median line is faintly indicated by a dark line in the basal half. The frons has two pairs of large setæ and two pairs of tiny setæ; the three posterior pairs, the last of which is the smallest though the first is also small, form a triangle, the first and last pairs being almost equidistant. The anterior or epistomal pair of setæ are large and are attached opposite the basal angles of the clypeus and some little distance from the antennal fossæ.

The epicranial areas are located on each side of the epicranial suture (Pl. 9, C-E). A pair of light lines depart from the frontal sutures and pass backward almost as far as the light median line of the epicranium, corresponding to adfrontal sutures which sometimes occur in the Rhynchophora. Each lobe of the epicranium bears setae as follows: One at each terminus of the rudimentary adfrontal suture; a small one opposite the middle of the frontal suture, and a longer one behind this almost equidistant from the epicranial suture; a long hair opposite the basal third of the frontal suture; one opposite the middle of the pleurostoma; one near the hypostomal angle of the mandible; one opposite the basal third of the hypostoma; one on disk behind this; and four tiny ones on the disk near the median basal angle of the lobe.

The antenna is a fleshy 2-jointed appendage located at the lateral angle of the frons (Pl. 9, D); the first joint is broad and short and bears one or more tiny hairs; the second joint is slender, finger-like, but short. The mandibles (Pl. 9, D, E) are very dark brown, bidentate, with median and basal hairs. The clypeus (Pl. 9, D) is attached in front of the frons and is basally margined with dark brown, but otherwise light in color. It bears four tiny hairs on the epistomal margin. The labrum (Pl. 9, D) is not so broad, is rounded in front, has a row of four setae in front of the middle, and is margined with setae. The maxillae (Pl. 9, D, E) are elongate, terminated by a 2-jointed palpus and a setose lacinia. They are provided with four setae, two near palpus and one near base. The stipes labii (Pl. 9, D, E) is triangular cordate, rather acutely angulate at base, bearing 2-jointed palpi at basal angles with a discal pair of setae and with several pairs of basal setae.

The body is glabrous except for the usual hairs found on each segment (Pl. 9, B). The prothorax is not divided dorsally on the anterior margin, which corresponds to the praescutum. There are six pairs of setae, the last of which occurs in the region of the alar lobe. Behind these on the scutal area are four pairs of hairs, the last of which occurs on the alar lobe. The mesothoracic spiracle occurs on a large lobe which causes an emargination of the prothorax and lies very close to the head. It is very elongate with a longitudinal slit. The mesothorax and metathorax dorsally consist of a spindle-shaped praescutum with a single pair of setae and the scutellum, extending from alar lobe to alar lobe and bearing only two pairs of hairs in the region of the alar lobe. The epipleurum of the mesothorax and metathorax bears a single hair. Each hypopleural lobe bears two setae. The sternum of the thorax consists of a median area or eusternum and two lateral lobes more or less connected medianly behind the sternum. The median portion is the sternellum and the lateral portions are the parasternal plates. Each thoracic sternum bears one pair of hairs, and each parasternum bears three pairs of hairs.

The first seven abdominal segments are normal, and each bears a very minute spiracle. In a fully matured specimen these segments grow larger to the fourth or fifth segment and then decrease in size. The seventh segment is the smallest of the normal segments. Dorsally each segment is transversely divided into four parts, praescutum, scutum, scutellum, and postscutellum. Each praescutum bears one pair of setae and each scutellum bears a small lateral pair. Each epipleural lobe bears two pairs of setae; and each hypopleural lobe is apparently longitudinally divided into two parts, the lower of which bears a single seta. Ventrally, each segment has two transverse lobes, the front one being the eusternum with the presternum depressed in front of it and the parasternum and lobe at each side. The second transverse area is transversely depressed and frontally consists of sternellum and poststernellum. There are no setae on the sternum of the abdomen. The eighth segment is dorsally greatly modified with a single pair of hairs on the praescutum and a single pair on the scutellar area, and with very elongated spiracles quite prominent (Pl. 9, F, G). Just outside of the spiracles on the epipleural lobe are two strong setae.

The dorsal face of the eighth segment is declivous (Pl. 9, B); the dorsum of the ninth segment is flattened and bears four pairs of setae, as shown in the figure (Pl. 9, F). The

dorsum of the ninth segment extends underneath, so that it is apical to the entire tenth segment. The tenth segment is completely ventral and very small. The tip of the abdomen showing the position of the tenth segment is illustrated in Plate 9, G.

PUPA (PL. 10)

Elongate, about 12 mm. long, white. This pupa is characteristically calandrid in the possession of very large thoracic spiracles located on a prominent lobe at the base of the prothorax (Pl. 10, B). The beak is very irregularly margined with numerous transverse depressions (Pl. 10, A). There are four pairs of large setæ and two pairs of tiny setæ on the head and beak. The four larger pairs of setæ are borne on tubercles, one on the head and three on the beak. The two pairs of tiny setæ are located medianly to the two basal pair on the beak, as shown in the drawing. The prothorax (Pl. 10, C) is rather elongate subquadrate, rounded in front, with basal angles rounded, and bears six pairs of setigerous tubercles, of which the apical pair are the largest. There are two antero-lateral, two postero-lateral, and one antero-median pairs of setæ. The mesothorax has one pair of scutellar setæ. The first six abdominal segments are normal, and each bears three pairs of scutellar setæ. The first six abdominal spiracles are larger and more prominent than the larval spiracles. The seventh and eighth spiracles are minute. The first two ventral segments are very much crowded. The seventh, eighth, ninth, and tenth segments are greatly modified both above and below. Dorsally the seventh segment is elongate, apically it is tuberculate, and it has two pairs of setigerous tubercles, one pair being on the larger apical tubercles. From a lateral view, it is seen that the seventh segment is dorsally the terminal segment, but ventrally it is surpassed by the other segments. In other words, it is laterally emarginate for the reception of the other segments, each of which includes the succeeding segment. The ninth segment is provided with a pair of very long, chitinous processes, corresponding to the cerci, at the side of which are two setigerous tubercles.

Ventrally (Pl. 10, A) the mesothorax is smallest, prothorax next, and metathorax next. The mesosternum is protuberant, the metasternum elongate and flattened. The coxæ are spherical; the femora are setigerous at the apex. The wing pads extend only to about the apex of the fourth abdominal segment.

ADULT (PL. 8)

Length 11 mm.; breadth at base of elytra 4 mm. Head small, spherical; beak separated from head by constriction, swollen in basal third, finely punctate in basal half; moderately curved, slender and cylindrical and smooth in apical half. Scrobes located in basal third beneath the swelling, oval, more approximate behind than in front. Gular suture extending almost entire length of venter of beak and head. Antennæ geniculate, scape almost as long as funicle. Funicle 6-jointed, first joint moniliform, succeeding joints more closely appressed, last joint very closely appressed to club. Club 2-jointed, basal joint occupying two-thirds of the length, shining, with a few minute hairs; apical joint spongy, short, and rounded at apex. Other funicular joints bearing a few tiny hairs. Eyes finely granulate, elongate oval, transversely contiguous beneath, anteriorly margined. Prothorax very long; moderately evenly punctate, with an irregular smooth median line indicated on disk; constricted near apex, apex tubular; narrowest at apex, roundly broadening to about the middle; sides almost parallel from middle. Scutellum small, subquadrate, moderately short, with slight humeral angles. Striæ moderately impressed, punctate. Intervals of irregular width, the first, third, and fifth being slightly wider than the alternate intervals, minutely punctate. Pygidium almost vertical, spongy, pubescent, with setigerous punctures. Undersides more sparsely punctate. Sternum flattened. Procoxæ and mesocoxæ cylindrical, metacoxæ oval, trochanters small, femora laterally

compressed and curved, ventrally inflated at middle, emarginate beyond this and bilobed at apex, thus forming a groove for the tibiae. Tibiæ moderately straight, grooved beneath and provided with a row of setæ on each side of the groove, apically curved downwards, terminating in a strong hook. Tarsi 4-jointed, first longer than broad, widest at apex, second about as long as broad, third about as long as first but broader at apex, emarginate for reception of fourth. Fourth elongate, curved, sub-cylindrical, armed with two curved, divergent claws. Intercoxal piece broad, angulate. First two abdominal segments connate at middle. Third and fourth segments about as long as second. Fifth segment longer, turned downward.

LIFE HISTORY

The female beetle having been fertilized enters between a leaf sheath and the stem and selects a spot for the deposition of an egg. The beetle then prepares a small cavity by means of the powerful mandibles located at the tip of the rostrum or beak. After having completed the cavity the beetle reverses its position and with the aid of the ovipositor deposits a single egg in the prepared place (fig. 1). On February 9, 1918, many

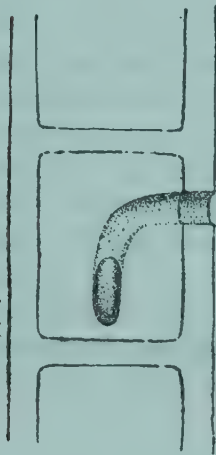


FIG. 1.—*Cosmopolites sordidus*: Section of sheath with egg *in situ* in compartment.

eggs were observed which were laid apparently a short time previously in the tissues, usually in the small compartments in the sheaths or stem. A few eggs were even found laid loosely in the slightly decayed leaf sheaths close to the healthy fleshy banana bulb, from which place they entered the bulb. The eggs, for the most part, are deposited singly in the sheaths near the crown at the surface of the soil. On hatching, the egg does not completely collapse. The larvæ eat their way in all directions in the bulb, and one can easily trace a channel as it gradually grows wider, terminating in a pouch near the outer surface in which the larva pupates on reaching maturity. The records for oviposition, hatching, and pupation are given in Table I.

TABLE I.—Egg and larval records of *Cosmopolites sordidus*, 1918

Egg No.	Egg deposited.	Egg hatched.	Larva pupated.
1.....	Feb. 10	Feb. 15	Mar. 2
2.....	...do.....	...do.....	Mar. 3
3.....	...do.....	...do.....	Do.
4.....	...do.....	Feb. 16	Mar. 2
5.....	...do.....	...do.....	Mar. 3
6.....	...do.....	Feb. 17	Mar. 4
7.....	...do.....	...do.....	Mar. 3
8.....	...do.....	...do.....	Mar. 6
9.....	...do.....	...do.....	Mar. 5
10.....	...do.....	...do.....	Mar. 6

From a few experiments the egg period was found to last from 5 to 7 days. From the character of the channels of the grubs it is the opinion of the writer that the eggs are deposited in the outer sheaths or between the outer sheath and the stem, the grubs working their way into the body of the bulb or trunk. The work of the larvæ is particularly destructive, since they girdle the plant in the immediate vicinity of the lateral roots put out from the bulb of the plant (Pl. 11, A), thus cutting off the passage of the sap. The larvæ not only work frequently in this region just described but may be found tunneling into the main trunk as far as the heartwood. The larvæ usually work below ground, but in a number of instances the writer has found them in the trunk as high as 2 feet above ground. The larval stage was found to last over a period of from 15 to 20 days. Due to the scarcity of material and to the fact that all infestations were gradually destroyed and cleaned up, the writer was unable to make further records on the seasonal habits of the species.

The larvæ upon attaining maturity construct an oval space at the end of the burrows, usually well toward the outer layers, where the larval head is cast, and where the larva pupates. The pupæ are naked. Jepson found in Fiji that a period of from 5 to 8 days from the time of pupation elapses before the emergence of the adult. The adults bear wings and are very sluggish. When disturbed they will "play 'possum" for a considerable length of time. The adults are gregarious and were found in clusters in cavities and depressions in the outer sheaths of the banana close to the surface of the ground and also below the surface. The length of life of the adult is not known. The writer has kept them in captivity without food for two months. Jepson in Fiji has kept the beetles in captivity about 14 weeks without food, and in the state of nature they undoubtedly will live longer. In all probability the banana root-borer continues to breed all the year round, provided that the food supply is plentiful. The beetles are nocturnal, only coming up from the soil at night for their activities above ground.

CONTROL

Since bananas are grown year after year on the same land and are produced from suckers springing from the parent plant, a plantation usually forms a breeding ground and nursery for these insects. The borer's mode of life renders it a difficult pest to control, as Knowles and Jepson (10) noted in Fiji. The egg, larval, and pupal periods are passed in or on the bulb of the banana or plantain. The adults apparently do not move far from the place where they have lived and developed so long as suitable food is available to attract the egg-laying females. In Java *Cosmopolites sordidus* is preyed upon and kept down by the larvæ of a listerid beetle and by those of some beetle of the family of Hydrophilidae. Jepson introduced these species into Fiji, where the banana root-borer is a serious

pest. Where banana plants are found infested in Florida and elsewhere in the States they should be destroyed immediately, and traps should be laid by using strips of healthy banana trunks. In Florida strips of banana plants proved more successful as a trap than did young plants on an infested piece of ground. As the beetles congregate under and about these strips they should be burned and the process repeated until the beetles are eradicated. It is very important that the traps be renewed, since the beetles are capable of living a considerable time without food.

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PLATE 8

Banana root-borer (*Cosmopolites sordidus*): Adult.



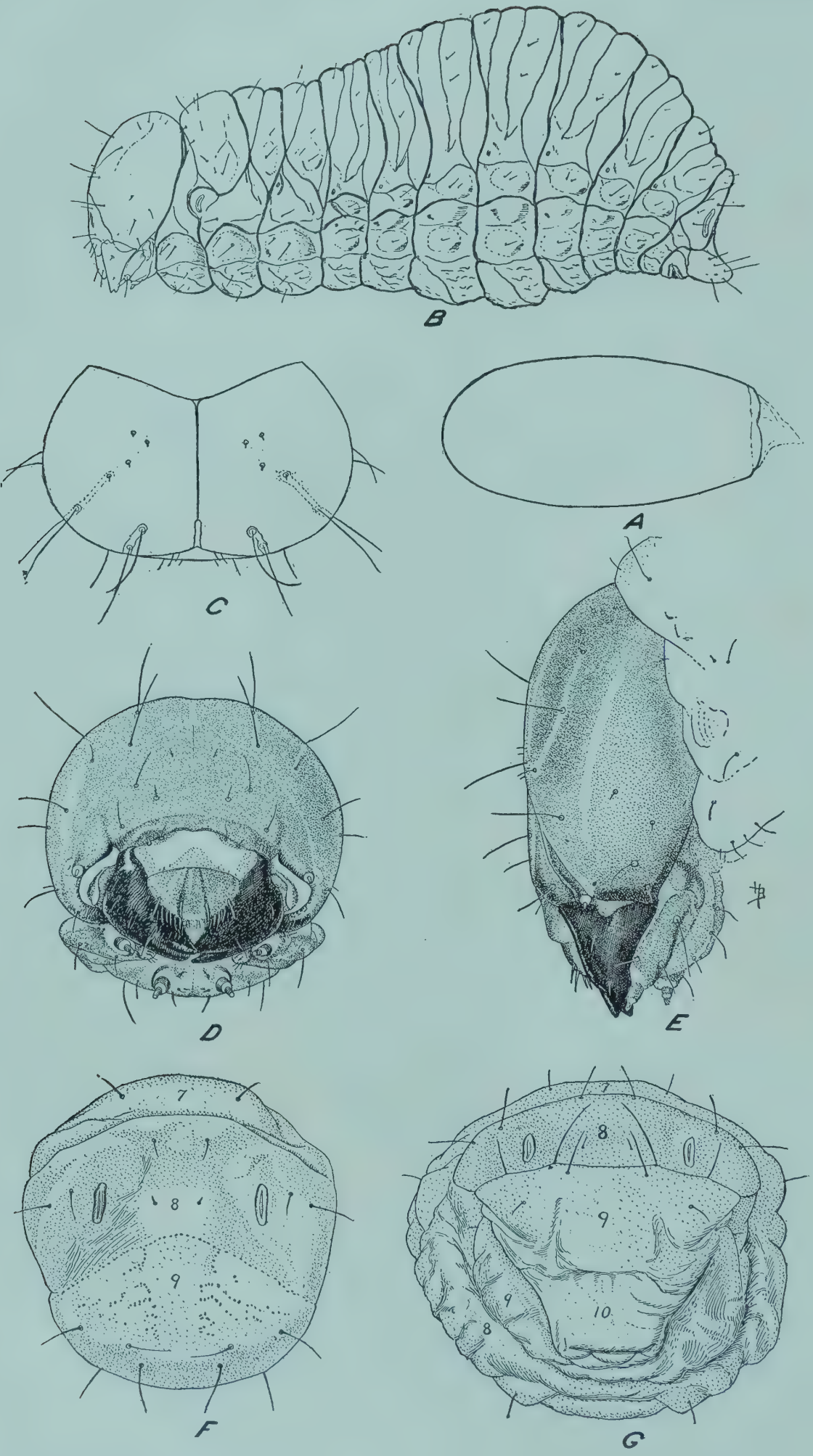


PLATE 9

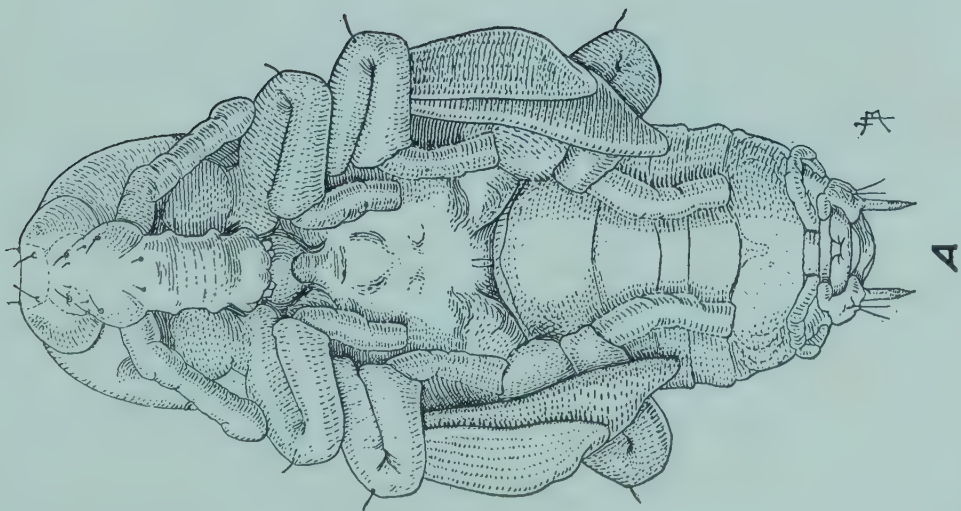
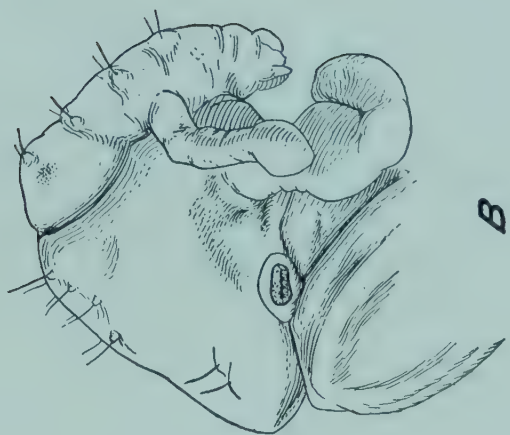
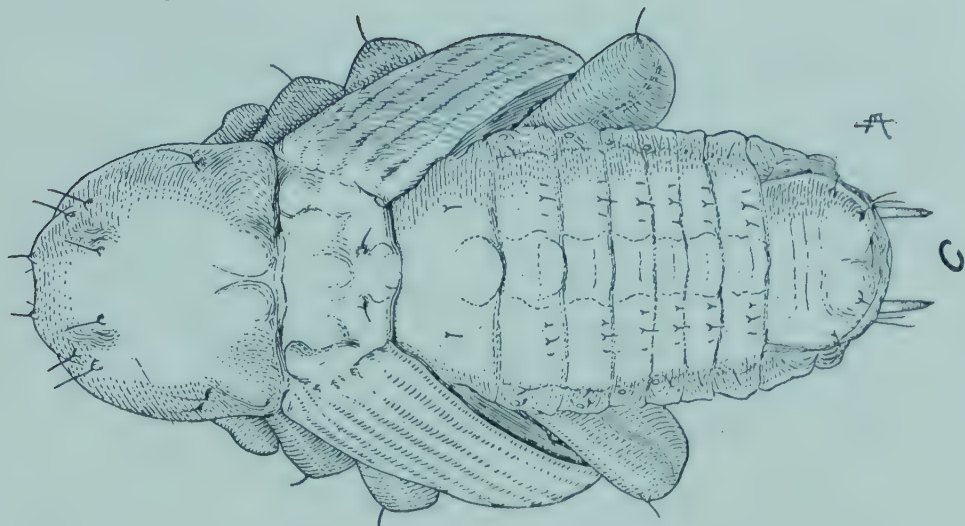
Egg and larva of banana root-borer:

- A.—Egg.
- B.—Larva, side view.
- C.—Head of larva, dorsal view.
- D.—Head of larva, face view.
- E.—Head of larva, side view.
- F.—Dorsal view of seventh, eighth, and ninth abdominal segments.
- G.—Posterior view of segments 7 to 10.

PLATE 10

Pupa and adult of banana root-borer:

- A.—Ventral view of pupa.
- B.—Lateral view of head and thorax of pupa.
- C.—Dorsal view of pupa.



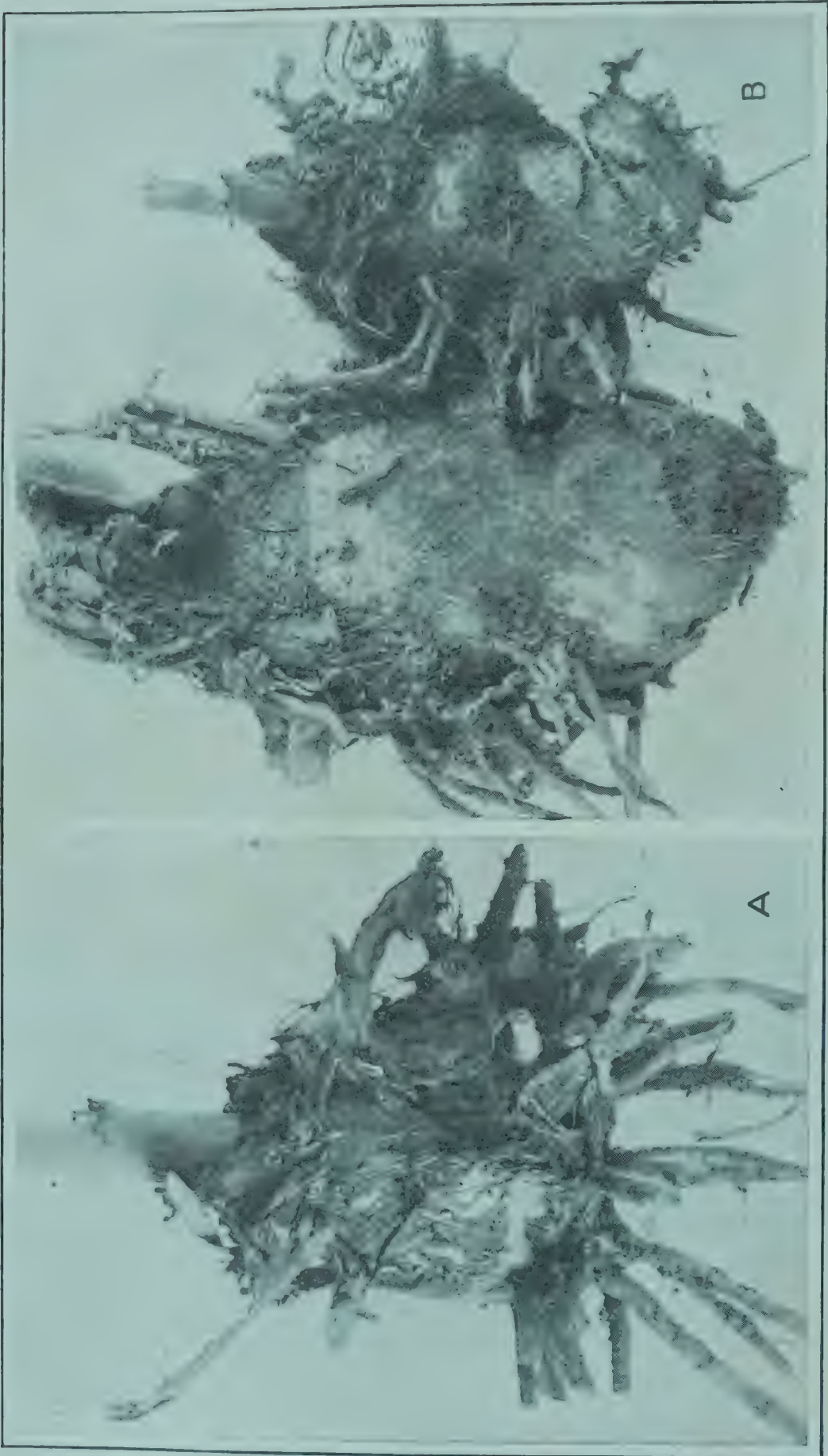


PLATE II

A.—Young healthy banana plant bulb with lateral roots.

B.—Young banana plant cut into, showing work of the larvæ of the banana root-borer. Illustration shows how lateral roots become severed by grubs working near roots.

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NO. 2

EFFECT OF CALCIUM SULPHATE ON THE SOLUBILITY OF SOILS

By M. M. McCool, *Professor of Soils*, and C. E. MILLAR, *Associate Professor of Soils*,
Michigan Agricultural Experiment Station

Additional information on the rate of formation of soluble salts in soils as affected by different factors is desirable. One phase of the subject of special interest is the immediate and residuary effects of fertilizing materials on soils. It seems that aside from its theoretical interest, such information should be of assistance in accounting for results that are obtained from the use of certain substances under field conditions. We have interviewed several of the earlier settlers in southern Michigan and have been informed by them that calcium sulphate was used rather freely by some farmers during the earlier stages of the State's agricultural development. The general impression of those whom we interviewed is that the application of calcium sulphate resulted favorably for a time, increasing the yields of small grains and clover, but later on failed to bring the desired results; hence this substance came to be looked upon as a soil stimulant. Some farmers are using it on wheat and clover, although the amount so consumed is relatively small. According to early reports, which are to be considered in a later publication, similar conditions existed in the agricultural regions of some of the eastern States.

Because of the experiences of the early agriculturists, the increasing interest in the fertilizing value of calcium sulphate, and the widespread use of acid phosphate, which contains appreciable amounts of the sulphate, it was considered advisable to investigate the effect of the sulphate both alone and in junction with calcium phosphate on the formation of soluble salts in soils, as well as the effect on the carbon-dioxid production. The freezing-point method was used to determine the former, and the titration method the latter.

METHOD OF PROCEDURE

In determining the effect of the chemicals on the rate of formation of soluble salts, 200 gm. of the soils in question were brought into contact

with 500 cc. each of distilled water and the substance in solution, and allowed to stand 24 hours with several thorough agitations. At the close of this period the mass was transferred to filter paper in large funnels. In some cases the soluble salts were reduced to a very low concentration by washing with distilled water, while in others the soils were removed, after drainage had ceased, but otherwise treated in the same way as in the former instances. The rate of formation of soluble substances in treated and untreated soils was determined under two sets of moisture conditions. The one which is here called low water content approximated the so-called optimum condition for plant growth; and the other, which is here called high water content, was secured by allowing 1 part of soil to 0.7 part of water and provided sufficient moisture to saturate the soil and leave a small column of about $\frac{1}{8}$ inch above it.

Soils of the following description were used in all the experiments:

Soil 1, a silt loam, light phase, containing a large amount of organic matter.

Soil 2, a heavy sand, rather low in organic content.

Soil 3, a fine sandy loam with a medium supply of organic matter.

Soil 4, a very fine sand containing a small amount of organic material.

Soil 5, a very heavy silt loam with a very high content of organic matter.

Soil 6, a silt loam well supplied with organic material.

EXPERIMENTAL RESULTS

The first series of experiments to be reported is the one in which the soils were treated with calcium sulphate, drained, and made up to the high water content, or 1 part of soil to 0.7 part distilled water. Treated and untreated portions of each of the soils studied were placed in jelly glasses, which were tightly covered and let stand in the laboratory. At about 4-day intervals they were thoroughly aerated by stirring, the covers being removed for one-half hour or more. The soils employed were air-dry and had been stored in the laboratory about 160 days. The results are set forth in Table I.

TABLE I.—*Effect of calcium sulphate on the solubility of unwashed soils held at high water content for various periods*

Soil No.	Condition of soil.	Freezing-point depressions.					
		After 2 days.	After 4 days.	After 6 days.	After 8 days.	After 10 days.	After 30 days.
		°C.	°C.	°C.	°C.	°C.	°C.
1	Treated.....	0.042	0.040	0.055	0.058	0.063	0.091
	Untreated.....	.003	.005	.008	.011	.013	.024
2	Treated.....	.044	.045	.050	.055	.059	.080
	Untreated.....	.000	.004	.005	.007	.009	.011
3	Treated.....	.043	.051	.054	.057	.058	.057
	Untreated.....	.002	.004	.006	.007	.008	.014
4	Treated.....	.045	.050	.050	.053	.055	.138
	Untreated.....	.000	.002	.004	.006	.010	.017
5	Treated.....	.046	.048	.051	.055	.058	.075
	Untreated.....	.003	.002	.004	.006	.012	.018
6	Treated.....	.045	.042	.050	.051	.052	.089
	Untreated.....	.008	.012	.012	.018	.024	.032

The effect of the calcium sulphate on the rate of formation of soluble salts in the soils investigated is appreciable. According to the data set forth in Table I, as well as other data not recorded, the reaction is rather gradual and prolonged. Of course the initial concentration of the solutions of the treated soils was high, and it is possible that this influenced the rate of changes which afterwards took place in the mass.

It was considered advisable to wash the soils until the concentration of the solution in the soils was at a very low point. This was done, and the series of tests with washed soils was carried on at the same time and under the same conditions as the previous one. The results obtained are presented in Table II. An examination of this table shows that the residuary effect of the calcium sulphate on the rate of formation of soluble substances in the soils is remarkable. The changes in the concentration of the soil solution did not all take place at once but continued for a number of days.

TABLE II.—Effect of calcium sulphate on the solubility of washed soils held at high water content for various periods

Soil No.	Condition of soil.	Freezing-point depressions.					
		After 2 days.	After 4 days.	After 6 days.	After 8 days.	After 10 days.	After 30 days.
		°C.	°C.	°C.	°C.	°C.	°C.
1	Treated.....	0.011	0.015	0.030	0.044	0.071	0.101
	Untreated.....	.003	.005	.008	.011	.013	.026
2	Treated.....	.002	.005	.012	.024	.057	.073
	Untreated.....	.000	.004	.005	.007	.009	.011
3	Treated.....	.000	.004	.010	.016	.028	.066
	Untreated.....	.002	.004	.006	.007	.009	.014
4	Treated.....	.000	.005	.018	.022	.058	.184
	Untreated.....	.000	.002	.004	.006	.010	.017
5	Treated.....	.001	.015	.028	.042	.052	.070
	Untreated.....	.003	.002	.004	.006	.012	.018
6	Treated.....	.000	.008	.013	.014	.024	.098
	Untreated.....	.008	.012	.012	.018	.024	.032

A clay loam soil was treated with the calcium-sulphate solution, washed, and let stand 30 days at the high water content, again washed until the freezing-point lowering of the solution in the soil was 0.005° C., and again let stand 30 days. At the end of this period the freezing-point lowering of the control or untreated soil was 0.040°, and that of the treated soil was 0.102°. The residuary effect of the treatment is quite persistent.

Another series was run in which the water content of the washed soils was lower, or approximately the so-called optimum point. The concentration of the solution in the soil was not determined until the end of a 30-day period. At that time the freezing-point lowerings of the soils were great and not strikingly different from those of the high water series. The results of this experiment are given in Table III.

TABLE III.—*Effect of calcium sulphate on the solubility of washed soils held at low water content for 30 days*

Soil No.	Condition of soil.	Freezing-point depressions.
		°C.
1	Treated.....	0.103
	Untreated.....	.015
2	Treated.....	.085
	Untreated.....	.010
3	Treated.....	.111
	Untreated.....	.013
4	Treated.....	.163
	Untreated.....	.007
5	Treated.....	.099
	Untreated.....	.023
6	Treated.....	.096
	Untreated.....	.023

Inasmuch as acid phosphate contains both calcium sulphate and calcium phosphate, a series was run in which the soils were treated with a saturated solution of calcium sulphate, a *N/10* calcium phosphate, and also a combination of the two. After treatment the soils were washed as in the series described above and let stand at the high water content 30 days. At the close of the period the concentration of the soil solution was determined by the freezing-point method. The results are given in Table IV.

TABLE IV.—*Effect of calcium sulphate and calcium phosphate alone and in combination on the solubility of soils after 30 days*

Kind of soil and treatment.	Freezing-point depressions.
	°C.
Sandy loam:	
Treated with calcium sulphate.....	0.134
Treated with calcium sulphate and calcium phosphate.....	.094
Treated with calcium phosphate.....	.028
Untreated.....	.035
Silt loam:	
Treated with calcium sulphate.....	.096
Treated with calcium sulphate and calcium phosphate.....	.084
Treated with calcium phosphate.....	.032
Untreated.....	.042

A glance at the data composing Table IV reveals that the calcium sulphate in the presence of the calcium phosphate is somewhat less active in changing the rate of solubility of these soils than it is when used alone. Moreover, where the calcium phosphate alone is added to the soils the solubility is somewhat lessened. This is in accord with the results reported by Bouyoucos.¹

¹ BOUYOUCOS, George J. RATE AND EXTENT OF SOLUBILITY OF SOILS UNDER DIFFERENT TREATMENTS AND CONDITIONS. Mich. Agr. Exp. Sta. Tech. Bul. 44, 49 p. 1919.

The results cited above immediately raise the question as to whether the great increase in concentration of the soil solution resulting from treatment with calcium sulphate is due to a stimulation of biological activities or to chemical reactions. To throw some light on this question experiments were undertaken in which the rate of production of carbon dioxid was measured. The method of procedure was as follows: The soils were allowed to stand over night in a saturated solution of calcium sulphate. They were then filtered and washed with distilled water until the concentration of the soil solution, when the soils were just saturated, was only a few parts per million. The soils were then allowed to dry. After thorough mixing, 60 gm. were weighed into 4-ounce bottles. The desired amount of water was then added and the bottles stoppered with rubber stoppers fitted with tubing so arranged that a current of air could be readily drawn through the soil. The bottles were stored in the dark at room temperature, and every 10 days the carbon dioxid was swept out by means of a current of air free from this substance, and the amount of carbon dioxid was determined. Samples of untreated soil were prepared in a similar manner and the carbon dioxid determined as outlined. Tables V and VI show the milligrams of carbon dioxid produced during the 10-day periods and also the total production for the 30-day period at the water contents used.

TABLE V.—Effect of calcium sulphate on the production of carbon dioxid at high water content

Soil No.	Treatment.	Carbon dioxid produced in—			Total carbon dioxid produced.
		10 days.	20 days.	30 days.	
		Mgm.	Mgm.	Mgm.	Mgm.
1	Calcium sulphate.....	3. 74	9. 24	7. 26	20. 24
	No treatment.....	8. 14	9. 02	5. 72	22. 88
2	Calcium sulphate.....	5. 06	7. 48	7. 26	19. 80
	No treatment.....	7. 26	8. 80	7. 04	23. 10
3	Calcium sulphate.....	5. 94	9. 90	8. 14	23. 98
	No treatment.....	7. 92	11. 88	9. 68	29. 48
4	Calcium sulphate.....	5. 28	7. 04	5. 94	18. 26
	No treatment.....	3. 74	7. 04	7. 04	17. 82
5	Calcium sulphate.....	9. 02	12. 76	13. 20	34. 98
	No treatment.....	5. 94	10. 34	8. 80	25. 08
6	Calcium sulphate.....	9. 24	12. 76	11. 88	33. 88
	No treatment.....	11. 00	13. 42	10. 56	34. 98

TABLE VI.—*Effect of calcium sulphate on the production of carbon dioxid at low water content*

Soil No.	Treatment.	Carbon dioxid produced in—			Total carbon dioxid produced.
		10 days.	20 days.	30 days.	
		Mgm.	Mgm.	Mgm.	Mgm.
1	Calcium sulphate.....	3.96	3.52	2.86	10.34
	No treatment.....	8.14	6.16	4.84	19.14
2	Calcium sulphate.....	4.40	3.30	3.30	11.00
	No treatment.....	7.81	5.06	4.40	17.27
3	Calcium sulphate.....	2.64	2.20	1.70	6.54
	No treatment.....	8.58	6.16	5.28	20.02
4	Calcium sulphate.....	3.30	2.64	2.42	8.36
	No treatment.....	5.50	3.52	2.86	11.88
5	Calcium sulphate.....	7.26	6.16	5.28	18.70
	No treatment.....	9.90	7.48	6.60	23.98
6	Calcium sulphate.....	6.38	5.72	4.62	16.72
	No treatment.....	10.78	7.26	5.28	23.32

At the high water content the production of carbon dioxid for the first 10-day period was depressed slightly in four soils by the treatment with sulphate, but in two soils it was stimulated. During the second period three of the untreated samples of soil still showed a slightly greater rate of production of carbon dioxid than the corresponding treated samples, and one of the treated samples of soil produced somewhat more of this material than the untreated. The remaining soils showed very slight differences in the production of carbon dioxid. During the third period there were more variations, two untreated samples producing more gas than the corresponding treated samples and three treated samples showing more activity than the untreated. The total production of carbon dioxid for the 30 days was greater for the untreated samples in four cases and less in one, and one soil showed practically no difference.

Without exception the untreated samples maintained at low water content showed a greater production of carbon dioxid for each period than the corresponding treated samples. In some instances the difference was so small as to be negligible, while in others it was very great. In every case the total production for 30 days was decidedly greater for the untreated samples.

It would appear from the data presented that the biological activities do not account for the changes in the solubility of the soils when treated with calcium sulphate, if the carbon-dioxid production may be taken as a measure. On the whole, there was a slight depression of such activities, especially when the samples were maintained at the low water content. This is somewhat at variance with the results reported by Fred and Hart,¹ who found an increased production of carbon dioxid from soil

¹ FRED, E. B., and HART, E. B. THE COMPARATIVE EFFECT OF PHOSPHATES AND SULPHATES ON SOIL BACTERIA. Wis. Agr. Exp. Sta. Research Bul. 35, p. 35-66, 6 fig. 1915.

containing 0.25 and 0.5 per cent calcium sulphate. It should be borne in mind, however, that the method of treating the samples was quite different from that in the experiment here reported. Several investigators have also reported a slight stimulation in ammonia production as a result of treatment with small amounts of calcium sulphate. In none of these experiments, however, were the soils thoroughly washed after treatment with the sulphate, and consequently it does not seem to be justifiable to make direct comparisons with our results.

At the expiration of 30 days the concentration of the soil solution of the samples maintained at the high water content was determined by thoroughly stirring the sample, withdrawing a portion to a freezing-point tube, and making the determination in the usual manner. Sufficient water was added to the samples maintained at the low moisture content to bring them up to that of the corresponding samples maintained at the high water content. The results of these determinations, together with the parts per million of soluble material, are presented in Tables VII and VIII.

TABLE VII.—Effect of calcium sulphate on the solubility of soils held at high water content for 30 days

Soil No.	Treatment.	Freezing-point depressions.	Soluble material.
		° C.	P. p. m.
1	Calcium sulphate.....	0.101	2,525
	No treatment.....	.026	650
2	Calcium sulphate.....	.073	1,825
	No treatment.....	.011	275
3	Calcium sulphate.....	.066	1,650
	No treatment.....	.014	350
4	Calcium sulphate.....	.184	4,600
	No treatment.....	.017	425
5	Calcium sulphate.....	.070	1,750
	No treatment.....	.063	1,575
6	Calcium sulphate.....	.098	2,450
	No treatment.....	.042	1,050

TABLE VIII.—Effect of calcium sulphate on the solubility of soils held at low water content for 30 days

Soil No.	Treatment.	Freezing-point depressions.	Soluble material.
		° C.	P. p. m.
1	Calcium sulphate.....	0.103	2,575
	No treatment.....	.015	375
2	Calcium sulphate.....	.085	2,125
	No treatment.....	.010	250
3	Calcium sulphate.....	.111	2,775
	No treatment.....	.013	325
4	Calcium sulphate.....	.163	4,075
	No treatment.....	.007	175
5	Calcium sulphate.....	.099	2,475
	No treatment.....	.023	575
6	Calcium sulphate.....	.096	2,400
	No treatment.....	.023	575

The total quantity of soluble material formed during the 30 days does not coincide with the amount of the carbon dioxid produced. The data show the treated samples to contain many times the amount of soluble material found in the corresponding untreated samples. There is one exception to this in the case of soil 5 at the high water content, where the treated sample contained only 175 parts per million more of soluble material than the untreated. It must be concluded, therefore, that the increase in soluble material takes place without the evolution of increased amounts of carbon dioxid and therefore is presumably due to other than biological agencies.

SUMMARY AND CONCLUSIONS

Six different soils were treated with a saturated solution of calcium sulphate. In one series of experiments the mass was transferred to filter paper, permitted to drain, and then transferred to containers and the rate of formation of soluble substances determined by means of the freezing-point method. The treatment was found to have increased the solubility of the soil to an appreciable extent.

In another series the amount of soluble material was reduced to a minimum by washing with distilled water, and the residuary effects of the treatment on the solubility were likewise determined. The calcium-sulphate treatment was found to have resulted in a very large increase in the rate of formation of soluble substances. The effects were great even when the soils were washed the second time. Obviously the treatment results in changes in the composition of the soil mass—in other words, a soil of different properties is formed. It seems that it is possible to alter the composition of the soil solution and that whether such change will have any effect on plant growth or not or whether the effect will be favorable or unfavorable will depend upon the nature of the soil and of the substances added. Moreover, it is probable that this phase of the subject has not received sufficient attention in connection with our field experiments.

Two soils of somewhat different texture and organic content were treated with a saturated solution of calcium sulphate, a *N/10* solution of calcium phosphate, and a combination of the two. The soils were washed, and the rate of formation of soluble salts was determined. The calcium sulphate markedly increased the solubility in each soil, while the calcium phosphate decreased the rate of formation of soluble substances. When calcium phosphate was used in conjunction with calcium sulphate, it counteracted the effects of the latter to some extent.

If the carbon dioxid produced, as determined by the methods used, is taken as a measurement of the biological activities, the increase in the rate of formation of soluble substances brought about by the calcium-sulphate treatment is due mainly to other causes.

FURTHER STUDIES ON THE INFLUENCE OF HUMIDITY
UPON THE STRENGTH AND ELASTICITY OF WOOL
FIBER ¹

By J. I. HARDY

Associate Wool Specialist, Wyoming Agricultural Experiment Station

INTRODUCTION

In a previous issue of the Journal the author published a preliminary report² of his work on the influence of humidity upon the strength and elasticity of wool fiber. An attempt was made to obtain a better method of testing wool in order that wool from sheep under various conditions of breeding, feeding, and range management might be satisfactorily tested. A study was also made upon the strength and elasticity of wool in an unscoured state under various conditions of humidity. A review of the literature was given in the earlier report and will not be repeated at this time.

EXPERIMENTAL WORK

After the work referred to above had been completed, further studies were begun upon scoured wool. As in the previous work, all samples were tested with a McKenzie fiber-testing machine. Wherever diameters are reported they are the results of measurements with a micrometer caliper unless otherwise stated. This micrometer had a ratchet stop and was graduated to read in hundredths of a millimeter. The micrometer was used in the lower jaw of the testing machine and had a small hand lens held stationary before it. With this arrangement it was possible to interpolate the readings to 0.001 mm. The diameters of the fibers were read at four different points. The smallest of these figures was in each case used in computing the tensile strength of the wool fiber.

Samples 991, 994, 996, and 997 had been extracted with ether and washed with hot water and tested at each of five relative humidities, 40, 50, 60, 70, and 80 per cent, when the operator was suddenly called into military service. The results of this work are given in Tables I and II.

TABLE I.—*Tensile strength of wool fiber at five different humidities*

Sample No.	Number of fibers.	At relative humidity of—				
		40 per cent.	50 per cent.	60 per cent.	70 per cent.	80 per cent.
		<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
991.....	100	279. 22	299. 47	289. 85	264. 29	258. 02
994.....	100	274. 77	280. 50	279. 73	255. 22	269. 59
996.....	100	295. 64	302. 00	281. 47	281. 40	271. 83
997.....	100	215. 34	210. 48	201. 87	200. 67	196. 56
Average.....	266. 24	273. 11	263. 24	250. 39	249. 00

¹ Approved for publication in the Journal of Agricultural Research by the Director of the Agricultural Experiment Station of the University of Wyoming.

² HARDY, J. I. INFLUENCE OF HUMIDITY UPON THE STRENGTH AND THE ELASTICITY OF WOOL FIBER. *In* Jour. Agr. Research, v. 14, no. 8, p. 285-296, 2 fig., pl. 48, 1918 Literature cited, p. 294-295.

TABLE II.—Elasticity of wool fiber at five different humidities

Sample No.	Number of fibers.	At relative humidity of—				
		40 per cent.	50 per cent.	60 per cent.	70 per cent.	80 per cent.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
991.....	100	27.80	29.08	28.48	29.24	30.04
994.....	100	28.64	30.92	31.08	31.32	34.20
996.....	100	34.32	38.32	38.28	40.36	37.40
997.....	100	24.20	25.28	27.28	27.00	26.48
Average.....	28.74	30.90	31.28	31.98	32.03

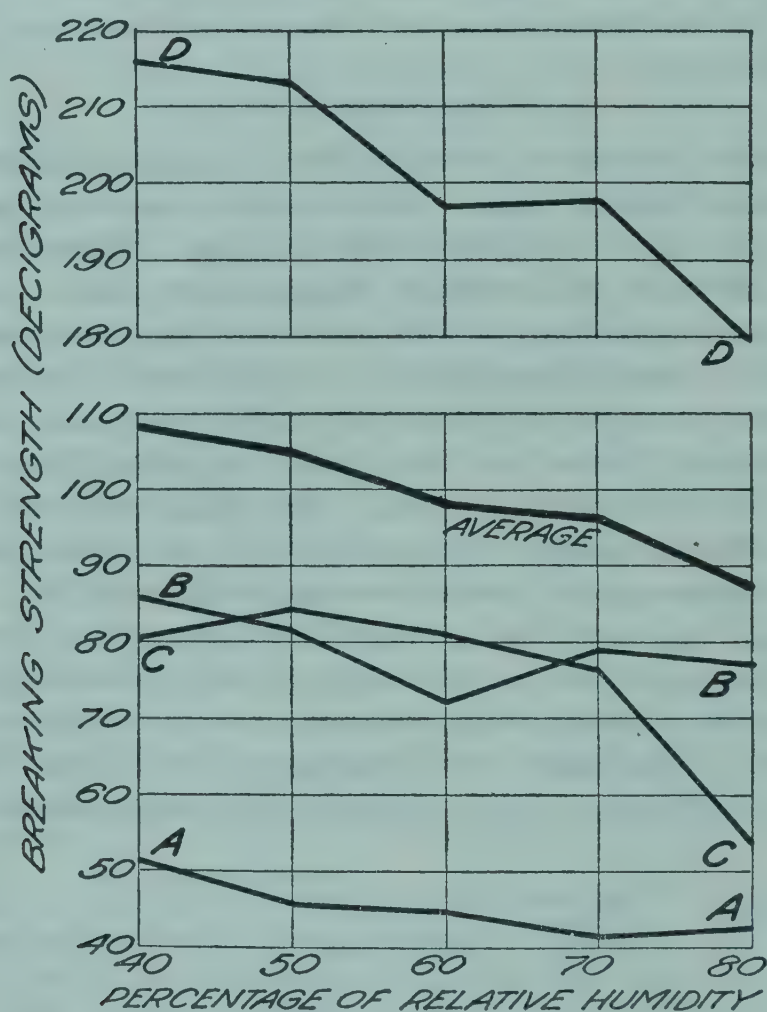


FIG. 1.—Graphs showing the effect of humidity upon the breaking strength of wool fiber.

Table I shows an average increase in the tensile strength of scoured wool as the humidity is raised from 40 to 50 per cent, and a decrease as the humidity is raised from 50 to 80 per cent. In Table II the average percentage of elasticity is shown to increase as the humidity is raised from 40 to 80 per cent.

A new operator was put upon the work in order to obtain more data under the same conditions and additional data on fibers of a smaller diameter. The diameter of the fibers of sample 991 averaged 0.016 mm.,

while samples 994, 996, and 997 had an average diameter of 0.026, 0.029, and 0.025 mm., respectively. There was one sample of wool with an average diameter of fibers less than 0.02 mm., and there were three samples with the average diameter above that figure.

The new set of samples chosen, A, B, C, and D, consisted of four samples with average diameters of 0.012, 0.018, 0.017, and 0.031 mm., respectively. Three of these samples were under 0.02 mm. in diameter, and one was larger. The range in average diameter of the fibers tested is from 0.012 to 0.031 mm. Fibers were tested from small locks of scoured wool from samples A, B, C, and D until 200 fibers were tested at each of

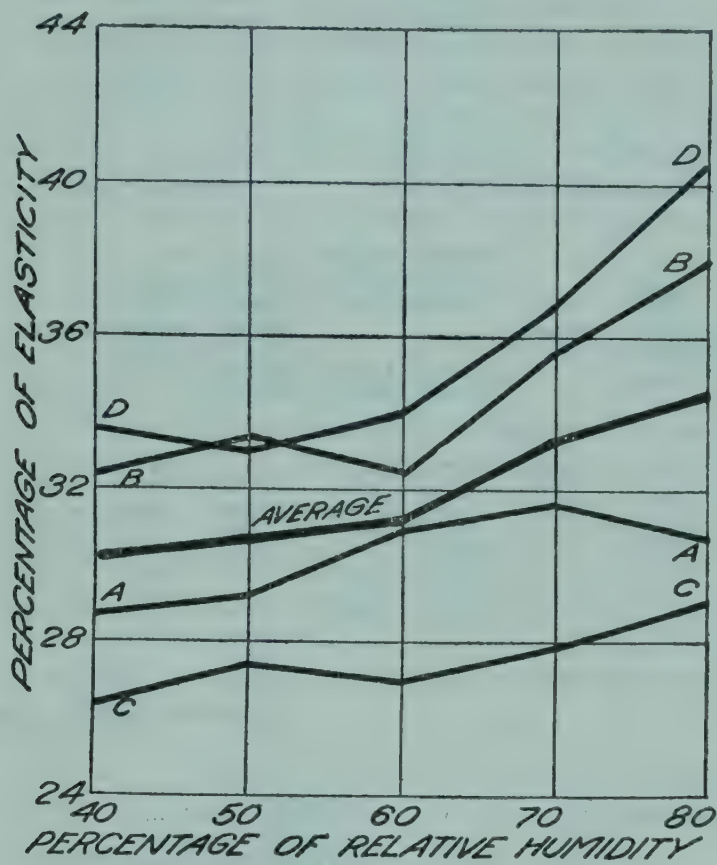


FIG. 2.—Graphs showing the effect of humidity upon the elasticity of wool fiber.

five humidities, as shown in Table III. It will be noted that the breaking strength of the fibers decreases quite uniformly as the humidity increases. Sample D shows a decrease in its tensile strength as the humidity increases up to 80 per cent, when there is a very slight increase. In A, B, and C the tensile strength seems to fluctuate up and down with no particular uniformity. These values for tensile strength were much more variable than those for the breaking strength. Several hundred additional fibers were tested on A, B, C, and D at humidities of 40 and 50 per cent, since the greatest variability seemed to occur at these two points. Graphs showing the values obtained on these samples of scoured wool for breaking strength and elasticity are shown in figures 1 and 2.

TABLE III.—Diameter, breaking strength, and tensile strength of scoured wool fibers at five different humidities

Sample No.	At relative humidity of 40 per cent.					At relative humidity of 50 per cent.				
	Diameter in thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Average.	Tensile strength per thousandth of a sq. mm.		Diameter in thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Average.	Tensile strength per thousandth of a sq. mm.	
				Average of 100.	Average of 200.				Average of 100.	Average of 200.
		Dgm.	Dgm.	Mgm.	Mgm.		Dgm.	Dgm.	Mgm.	Mgm.
A.....	11.9	48.59	48.56	433.97	396.65	10.79	47.67	45.39	521.67	466.88
	13.2	48.52	359.33	11.54	43.10	412.08
B.....	20.04	86.62	89.33	274.37	279.49	17.86	82.81	86.17	330.37	324.87
	20.34	92.04	284.60	18.83	89.52	319.36
C.....	19.89	88.03	85.28	283.29	301.96	17.40	82.89	81.21	349.05	341.80
	17.44	82.52	320.63	17.40	79.53	334.54
D.....	30.78	208.02	209.68	281.50	280.94	32.57	221.86	214.54	266.54	267.64
	30.48	211.32	280.38	31.36	207.22	268.74

Sample No.	At relative humidity of 60 per cent.					At relative humidity of 70 per cent.				
	Diameter in thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Average.	Tensile strength per thousandth of a sq. mm.		Diameter in thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Average.	Tensile strength per thousandth of a sq. mm.	
				Average of 100.	Average of 200.				Average of 100.	Average of 200.
		Dgm.	Dgm.	Mgm.	Mgm.		Dgm.	Dgm.	Mgm.	Mgm.
A.....	11.84	44.89	44.90	407.40	379.74	10.80	41.09	40.31	455.25	432.26
	12.75	44.91	352.07	11.09	39.53	409.27
B.....	16.44	72.85	72.30	343.30	336.41	18.85	85.86	79.97	324.14	329.28
	16.55	71.74	329.51	16.85	74.08	334.41
C.....	18.37	81.02	81.02	305.68	305.68	16.57	74.62	76.93	347.76	346.77
						17.08	79.23	345.78
D.....	30.65	197.02	197.02	266.27	266.27	31.26	199.67	196.49	260.07	263.64
						30.35	193.31	267.20

Sample No.		At relative humidity of 80 per cent.				
		Diameter in thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Average.	Tensile strength per thousandth of a sq. mm.	
			Dgm.	Dgm.	Mgm.	Mgm.
A.....		11.97	45.41	42.95	403.85	437.92
		10.45	40.48	471.98
B.....		17.26	73.79	79.94	315.37	315.05
		18.64	86.08	314.72
C.....		13.62	51.46	53.78	353.21	343.32
		14.65	56.10	333.42
D.....		30.02	183.75	179.79	259.60	265.92
		28.69	175.83	272.24

The heavy line shows the average values obtained for all the results secured at each humidity. The average breaking strengths of these samples of scoured wool decrease as the humidity increases, while the elasticity shows an increase with an increase in humidity.

The wide variations in the values for tensile strength as compared with similar values for breaking strength led the writer to compare the tensile strengths of fibers of different diameters in locks of wool A, B, and D.

Graphs showing the variation in the tensile strengths of three different samples of wool are shown in figure 3 in the curves A-A, B-B, and D-D.

The fibers tested in these curves range from 0.008 to 0.038 mm. in diameter. The number of fibers tested at each humidity varies considerably. In some cases only 30 or 40 were tested, while in other cases as many as 250 of a given diameter were tested. Sample A of curve A-A ranges in fineness from 0.008 to 0.018 mm. The tensile strength decreases from 667 to 260 mgm. per thousandth of a square millimeter at the lowest point. Sample B shows a decrease from 466 mgm. at 0.01 mm. to 315 mgm. at 0.022 mm. The curve of sample B follows that of sample A very closely from a diameter of 0.01 mm. to one of 0.018 mm. and rises slightly from a diameter of 0.018 mm. to one of 0.022 mm. Sample D decreases from 320 mgm. at 0.023 mm. to 232 mgm. at 0.038 mm.

These curves show that the tensile strength of wool decreases with an increase in diameter. The drop is most abrupt with the sample of fine wool. The coarsest sample has the most gradual drop in its diameter and tensile strength curves. If the breaking strength of wool varied directly as the area of cross section, the curve would follow the line E-E. If the breaking strength varied as the diameter or circumference, the tensile strength curve would follow the line F-F. The curve for the tensile strength of sample D follows the line D-D and lies between these two lines E-E and F-F. This fact seems to indicate that the breaking strength of medium and coarse wool varies with some power of the diameter which lies somewhere between the first and second.

For fine wool like sample A, a curve showing the strength of the wool very closely follows a curve plotted with 1 , or any constant, and the first power of the diameter. This fact indicates that the breaking strength of fine wool does not vary directly with the area of the cross section but with a value which is very close to the first power of the diameter. Curve C-C shows the relation between the tensile strengths and diameters of wool fibers obtained from data published by Hill.¹ In the present experiment, 1,000 fibers were broken to obtain the points in this curve, and each diameter was measured after breaking as nearly as possible at the point of breakage. This curve also follows very closely the curve F-F. By inspecting the graphs it is easy to see that the widest variations in the curve F-F plotted from $\frac{1}{D}$ are found at the smallest diameters. As this curve approaches the larger diameters it tends to become rather flat.

In the first three samples of Table III there is a large variation between the largest and smallest tensile strengths of the wool fibers of those samples. When fibers are tested with such a wide variation in their tensile strength as is found in locks of fine wool, it is necessary that these fibers be carefully mixed in order to get satisfactory results. There is a tendency for an operator to pull the largest fibers in fine wool, while with

¹ HILL, J. A. STUDIES ON THE STRENGTH AND ELASTICITY OF THE WOOL FIBER. I. THE PROBABLE ERROR OF THE MEAN. *In* Wyo. Agr. Exp. Sta. 21st Ann. Rpt., 1910-11, suppl., 139 p. 1911.

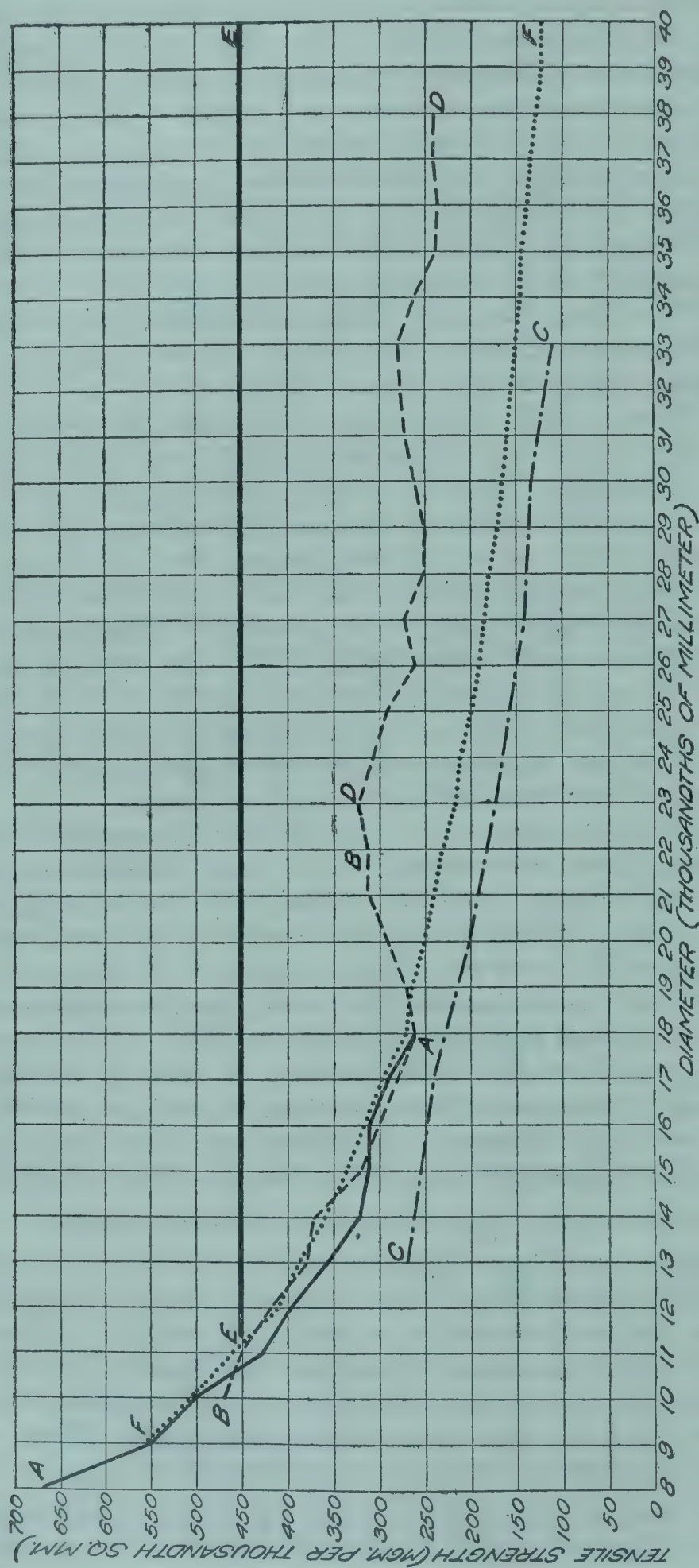


FIG. 3.—Graphs showing the relation between the diameter and the tensile strength of wool fiber.

coarser samples there is not such a tendency. This fact and the fact that larger fibers can be more accurately measured with a micrometer caliper make it possible to get satisfactory results for tensile strengths with samples of coarser wools. Then again the coarser wools have breaking strengths which vary more closely with the areas of the cross section of the wool than do the breaking strengths of fine wool samples, as is shown in F-F of figure 3. The coarse wools may be measured from the original lock, and their breaking and tensile strengths may be determined quite satisfactorily.

Sometimes it is necessary to make the closest possible comparison of the effects of various conditions or chemical reagents on a given grade of wool, as in the case at hand. The writer desired to determine the effects of various humidities upon a uniformly mixed sample of wool. Single fibers were drawn from sample B and placed consecutively in six different groups, numbered 1 to 6, with their ends extending from one piece of adhesive tape to another which was laid parallel to it and about 2¾ inches from it. Always beginning with No. 1, these fibers were placed one at a time in each of these six groups until 100 fibers, or the desired number, were in each of the six small locks. By making five series of these groups and subjecting the same numbers of each group to the same test, it is possible to get some very satisfactory comparisons. Although it is very tedious work, these fibers may be picked out by hand at the rate of 200 an hour. Five small locks, each containing 120 fibers, were tested in the scoured condition at humidities of 40, 50, 60, 70, and 80 per cent and saturated. Similar locks were scoured with ether and hot water and tested under the same conditions. The saturated fibers were kept between moist filter papers until tested.

Table IV and figure 4 show the results of this experiment.

TABLE IV.—Elasticity and breaking strength of scoured and unscoured wool from sample B

[Average of 600 fibers]

Percentage of humidity.	Scoured wool.		Unscoured wool.	
	Elasticity.	Breaking strength.	Elasticity.	Breaking strength.
	Per cent.	Dgm.	Per cent.	Dgm.
40.....	25.80	65.14	26.40	69.06
50.....	30.76	64.11	31.48	68.97
60.....	33.96	64.64	34.72	67.01
70.....	37.08	59.53	38.00	63.80
80.....	40.08	60.10	43.64	60.44
Saturated.....	33.76	59.16	34.60	63.42

The curve for unscoured wool shows that the breaking strength decreases as the relative humidity changes from 40 to 80 per cent and increases when the wool becomes saturated. In scoured wool the curve is more irregular. There is a definite drop as the humidity changes from

40 to 80 per cent, although the curve makes almost a straight line from 70 per cent up to the point of saturation. The elasticity curves for scoured and unscoured wool are nearly parallel, rising as the humidity changes from 40 to 80 per cent and falling from this point to that of saturation.

SUMMARY

(1) The tensile strength of wool increases with the decrease in the diameter of the wool fiber.

(2) Fine wool has a breaking strength varying more closely with the first than with the second power of the diameter.

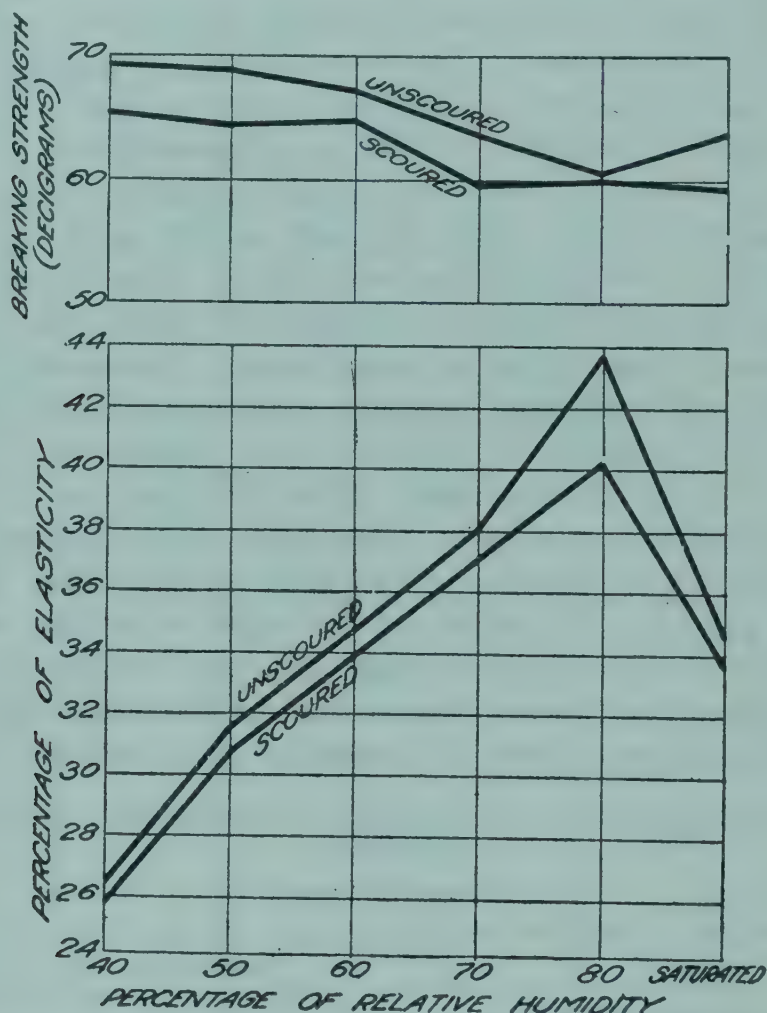


FIG. 4.—Graphs showing the effect of humidity upon the breaking strength and elasticity of wool fiber.

(3) Coarse wool has a breaking strength varying with a figure which lies somewhere between the first and second powers of the diameter.

(4) It is necessary to mix samples of fine wool carefully before testing in a testing machine if the best results are to be obtained.

(5) The breaking strength and tensile strength of both scoured and unscoured wool decrease with an increase in relative humidity from 40 to 80 per cent and show a tendency to increase from this point to that of saturation.

(6) The elasticity of scoured and unscoured wool increases with an increase in relative humidity from 40 to 80 per cent and decreases from this point to that of saturation.

COMPOSITION AND DENSITY OF THE NATIVE VEGETATION IN THE VICINITY OF THE NORTHERN GREAT PLAINS FIELD STATION

By J. T. SARVIS

Assistant in Dry-Land Agriculture, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The grazing industry in the Northern Great Plains area is intimately concerned with the composition and density of the native vegetation. This paper deals with the native vegetation as it exists at present in the section under consideration. While parts of the discussion will apply in general to the Great Plains area, it pertains to western North Dakota and in particular to the territory adjacent to the Missouri River on the west near Mandan. This point lies practically on the one hundred and first meridian and just south of the forty-seventh parallel, north latitude. The Bureau of Plant Industry has one of a number of field stations located here under the direction of the Office of Dry-Land Agriculture. One of the lines of investigation in connection with this station is a grazing experiment in cooperation with the North Dakota State Experiment Station. This investigation is primarily concerned with determining the carrying capacity of the range in that section and working out a grazing system adapted to conditions in the Great Plains. In connection with this work it is necessary to make detailed studies of the native vegetation in order to observe any changes that may occur in the structure of the plant cover. These studies have furnished the material of this paper.¹

TOPOGRAPHY AND SOIL

The topography of the area around Mandan varies from rolling to nearly level. The land is cut by numerous ravines and coulees, which drain into the Heart and Missouri Rivers. The altitude of the field station is approximately 1,700 feet above sea level.

The following description of the soil of this area is quoted from "The Story of the Prairies" by Willard (9),² formerly geologist at the North Dakota Agricultural College:

A belt having an indefinite edge to the westward lies along the west side of the Missouri River, which belt represents the western limits of the glaciated area of North Dakota, and of the Continent of North America. This "belt" of land along the west

¹ The annual reports by the author of the cooperative grazing experiment at Mandan have been frequently referred to and used in the preparation of this paper. These reports are on file in the Office of Dry-Land Agriculture, the North Dakota Agricultural College, and the Mandan Field Station.

² Reference is made by number (*italic*) to "Literature cited," p. 71-72.

side of the river shows by the character of the soils and the rocks that lie upon or near the surface that the great continental glacier was once here. Toward the west the belt fades out and becomes indistinguishable from the land farther west over which the ice did not pass, but the eastern part of the belt is sufficiently modified as to the soils and the landscape features to be readily recognized.

The soils, therefore, in the belt bordering the Missouri River on the west constitute a transition type from the glacial soils of the eastern portion of the State to the non-glaciated or residual soils of the southwestern portion of the State.

CLIMATE

The United States Weather Bureau Station at Bismarck has made continuous meteorological observations since 1875. Bismarck is located on the east side of the Missouri River, only about 5 miles distant from Mandan. Observations were begun at the Mandan Field Station during 1913. From 1875 to 1914, inclusive, or 40 years, the mean annual precipitation was 17.41 inches. The greatest annual amount during this period was 30.92 inches in 1876, while the lowest was 11.03 inches in 1899. During 1917 the record at the Mandan Field Station was 10.31 inches. The mean seasonal precipitation from April 1 to July 31, inclusive, was 9.91 inches during the 40-year period. The month of maximum precipitation is June, with a mean of over 3.5 inches, and the month of minimum precipitation is February, with less than 0.5 inch.

The temperature is extreme in both winter and summer. The lowest recorded to date was 45° F. below zero in January, 1916, while the highest was 107° above zero in July, 1910 and 1917. The average dates of killing frosts in spring and autumn are about May 15 and September 15, respectively, but frosts have occurred as late as June 7 and as early as August 23. The average frost-free period is 128 days. The prevailing wind direction is from the northwest. The average wind movement near the ground is about 6 miles per hour.

PLANT FORMATION

According to a map of "Plant Formations of the United States," by Shantz and Zon,¹ this region would come within the "short-grass formation." However, Dr. F. E. Clements, who visited the field station during the summer of 1917, is of the opinion that it would be more properly placed in the "long-grass" or "prairie formation," because of the long grasses and other plants which are typical of a prairie formation. From actual determinations in the field the percentages of short-grass and long-grass cover have been found to be nearly equal, so that the formation could be put in either class, according to the viewpoint of the observer. If the secondary plant layer is considered as the determining factor, the region falls in the long-grass formation. The vegetation in this particular area might be considered as in a transition zone, since the dominating species are typical of both formations.

¹ SHANTZ, H. L., and ZON, R. PLANT FORMATIONS OF THE UNITED STATES. Paper presented before the Ecological Society of America at its annual meeting in New York in 1916. The map will appear in the Agricultural Atlas.

The dominating species are *Bouteloua gracilis* (*B. oligostachya*) and *Stipa comata*, which form a distinct association. This is an association composed of *Bouteloua gracilis*, which is typical of the short-grass formation, and *Stipa comata*, which is a typical long-grass species. This association is dominated by the *Bouteloua*. Sarvis¹ has described in a paper other sections of western North Dakota which show the same dominating species.

COMPOSITION OF THE VEGETATION

In Plate 12 is illustrated the general character of the vegetation on the prairie in the Mandan region. In 1915, when this photograph was taken, the season was very favorable, and all plants reached a maximum development. The composition of the vegetation is thus very clearly illustrated.

In the following list of plants the arrangement of species is in the order of abundance. The order of the primary and secondary species is subject to slight modifications as the studies are extended. The order of the dominant species was determined by measurements from quadrat maps and in the field. The order of the primary species, other than grasses, was determined by count. The secondary species are listed in the estimated order of their abundance.

DOMINANT SPECIES

<i>Bouteloua gracilis</i>	<i>Carex filifolia</i>
<i>Stipa comata</i>	<i>Carex heliophila</i>

PRIMARY SPECIES

<i>Artemisia gnaphalodes</i>	<i>Artemisia frigida</i>
<i>Koeleria cristata</i>	<i>Stipa viridula</i>
<i>Solidago pulcherrima</i>	<i>Eschinacea angustifolia</i>
<i>Agropyron smithii</i>	<i>Aristida longiseta</i>
<i>Artemisia dracunculoides</i>	<i>Polygala alba</i>
<i>Psoralea argophylla</i>	<i>Stipa spartea</i>
<i>Andropogon scoparius</i>	<i>Ratibida columnaris</i>

SECONDARY SPECIES

<i>Muhlenbergia cuspidata</i>	<i>Aster multiflorus</i>
<i>Lacinaria punctata</i>	<i>Petalostemon purpureum</i>
<i>Calamovilfa longifolia</i>	<i>Petalostemon candidum</i>
<i>Agropyron caninum</i>	<i>Lactuca pulchella</i>
<i>Bouteloua curtipendula</i>	<i>Vicia sparsifolia</i>
<i>Comandra pallida</i>	<i>Agropyron tenerum</i>

The grasses, other than the dominant species, are in the estimated order of abundance. It is difficult to make individual counts of them, since they usually occur in bunches. If bunches or mats were considered as single plants and enumerated as such the number would have no significance when compared with that of other plants which usually occur as individuals.

¹ SARVIS, J. T. NATIVE GRASSES OF WESTERN NORTH DAKOTA. Paper presented before the Ecological Society of America at its annual meeting in New York in 1916.

When the vegetation is considered from the standpoint of grazing, only a very few species are important factors in the total amount of forage annually produced. Sampson (6) has discussed this point more fully. In this region, *Bouteloua gracilis* and *Stipa comata* are the most important species, both on account of their total forage production and their value as grazing grasses.

The value of a given species for grazing purposes depends upon (1) its abundance, (2) whether it is relished by stock, (3) its length of growing season, (4) its ability to withstand trampling and to recover readily from grazing, and (5) its adaptation to drought conditions. According to these requirements, *Bouteloua gracilis* would take first rank and *Stipa comata* would be second in importance.

A plant may be of importance in relation to grazing because of its abundance, whether it is or is not of grazing value. If it is a valuable grazing species it is of primary importance, and if it is of minor grazing value it is of importance because it occupies ground surface that might otherwise support a more valuable species. On the other hand, a species may be greatly relished by stock, as *Andropogon furcatus* at Mandan, but occur in such limited areas that it is unimportant in the total amount of forage annually produced. In pastures where this grass occurs it is cropped close to the ground throughout the season, as illustrated in Plate 13, A.

Bouteloua gracilis is grazed with avidity at all times of the year. It cures well on the ground without great loss of its nutritive value, and late in the fall cattle eat it in preference to any other grass. Although *Stipa comata* has the disadvantage, for a short period, of its sharp needles, it is so much more abundant than other species, except *B. gracilis*, that it enters largely into the feed of grazing animals. It is the first grass to produce green shoots in the spring, and it usually produces more growth late in the fall than do other species.

A grass that is similar in appearance and often confused with *Bouteloua gracilis* is *Bulbilis dactyloides*, or buffalo grass. It has a better reputation for grazing and is more widely known by a popular name than any other single species of grass in the Great Plains. However, out of several thousand acres of native vegetation surrounding the field station, there are less than 5 acres of the true buffalo grass. On a trip over western North Dakota in the summer of 1916, the author found this grass in only a few small areas. Blue grama (*Bouteloua gracilis*) is and always has been called buffalo grass by the people in the Great Plains area. This misnomer has been and is so universal that it is difficult to obtain reliable information concerning the abundance and importance of buffalo and blue grama grasses for grazing in the early history of the range. However, at present the true buffalo grass occurs only in small amounts in this region and in western North Dakota, where it is evident it never

was as abundant as in western South Dakota. Pound and Clements (4) said in regard to buffalo grass:

The buffalo-grass was, until recently, supposed to have once covered the greater portion of Nebraska; its disappearance has, as a matter of sentiment, been connected with that of the buffalo. The patches of buffalo-grass, which are found scattered here and there over the State, are to be regarded as intrusions rather than stragglers left by a retreating species.

Griffiths (2) says in regard to *Bulbilis dactyloides*:

Bouteloua gracilis, especially when not in head, is very similar and frequently mistaken for it. On this account the true buffalo grass is very much overestimated in importance, because there are so many things included with it in the popular mind. Much of the credit given this species is due to the grammas, which in age especially look much like it. On the other hand, the species is an important one throughout its range.

In southwestern South Dakota, at the Ardmore Field Station, where a grazing experiment is now being conducted, the important grazing grasses are *Bulbilis dactyloides*, *Bouteloua gracilis*, and *Agropyron smithii*. This association is dominated by the *Bulbilis*.

It often happens that a species that is of little grazing value in one section is of value in another area. For example, *Aristida longiseta* is of little grazing value at Mandan, since it is the last plant that cattle will take even when the pasturage is short, as illustrated in Plate 13, B. However, in other sections, Griffiths, Bidwell, and Goodrich (2) report this species as being of considerable value.

Some species are indicators of overgrazing, as *Artemisia frigida* at Mandan. In pastures where this plant occurs in abundance it usually will be found that the area has been overstocked for several seasons.

In the vegetation of this area no poisonous plants are abundant enough to be harmful. However, in areas farther west in North Dakota, the common "loco weed" (*Oxytropis lamberti*) is abundant and causes serious losses of stock in certain seasons.

All the plants mentioned in the list on page 65 enter more or less into the feed of grazing animals, but, as noted, only a few species produce a considerable percentage of the total forage. One of the reasons for this fact is the inability of many plants to produce more than a limited second growth after they have once been removed by grazing.

DENSITY OF VEGETATION

In a consideration of plant density in relation to grazing problems it is desirable and necessary to make clear and concise distinctions between frequently recurring terms. Plant density should refer to the "stand" or thickness of plants upon the ground surface. The ground surface is the total area of land under consideration, whether vegetated or unvegetated. Bare ground should be understood to refer to the un-vegetated portion of the ground surface or the spaces in the cover between

individual plants or between mats and bunches of species which grow in that manner. The term cover (8), or ground cover (5), is frequently and conveniently used in connection with discussions of vegetation. However, when the term cover is applied in connection with grazing investigations it should be defined, for it may mean one of two things: (1) basal cover, or the ground surface limits of living vegetation, or (2) the foliage cover, which is the plant layers above the basal cover. When the foliage cover is removed, as by close grazing or clipping, the basal cover remains. Plant layers as described by Clements (1) are vertical zones based on the height of plants. On the prairie around Mandan two layers are important—the ground layer, as *Bouteloua* and *Carex*, and the secondary layer, as *Stipa* and *Psoralea*.

Species that grow in mats or in bunches are most accurately expressed in terms of basal cover. For example, *Bouteloua* basal cover would refer to the amount of ground surface actually covered by *Bouteloua* if the foliage were removed by grazing or clipping. In such species it is possible to make the determinations with almost mathematical precision. Species that occur as individuals are best expressed in terms of their abundance per unit area. Shantz (7) says in regard to this point:

Those species which form mats can not be well represented in numbers per square meter, and on this account the percentage of surface covered is given instead.

The foregoing statements in regard to basal and foliage cover are very clearly illustrated in Plate 14. In 1915 the foliage cover was very

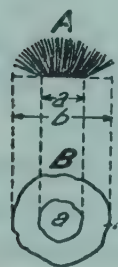


FIG. 1.—Diagram of grass mat: A, from side; B, from above. a, Basal cover; b, foliage cover.

heavy because growth conditions were favorable and the area had not been grazed. An estimate of the total cover based upon the amount of foliage cover could easily have been made at that time. But in 1916 on the same area, with the foliage cover removed, there would have been no basis for comparison with the 1915 condition. This illustrates the undesirability of utilizing the foliage cover, under all conditions, as a basis for estimating the possibilities of forage production and the consequent carrying capacity. A clear distinction between basal cover and foliage cover is, therefore, necessary and important.

The two illustrations of Plate 14 picture the same area, but one illustrates a heavy foliage cover and the other only the basal cover. However, the potential ability of the area to produce under similar conditions as heavy a foliage cover as in 1915 is unchanged.

Figure 1 illustrates the difference between the basal cover and the foliage cover. The limit of basal growth is *a*, while the limit of foliage growth is *b*. In a given case the surface area of the foliage cover is greater than that of the basal cover, yet the amount of forage is the same. The basal cover is more permanent than the foliage cover, since the latter may be readily removed by grazing. The quadrat map (fig. 2) in the 30-acre pasture, which was mapped in 1915 and remapped in 1916, shows,

with the exception of a few annual species, the basal cover to be practically the same in both years. If the maps had been drawn on the basis of the foliage cover, there would have been a great difference between the 1915 and 1916 maps. The photographs illustrate this difference more clearly than would be possible by quadrat maps. But if the maps are drawn on a basis of the basal cover, various maps of a given quadrat would show actual changes as they occur from grazing. This is really

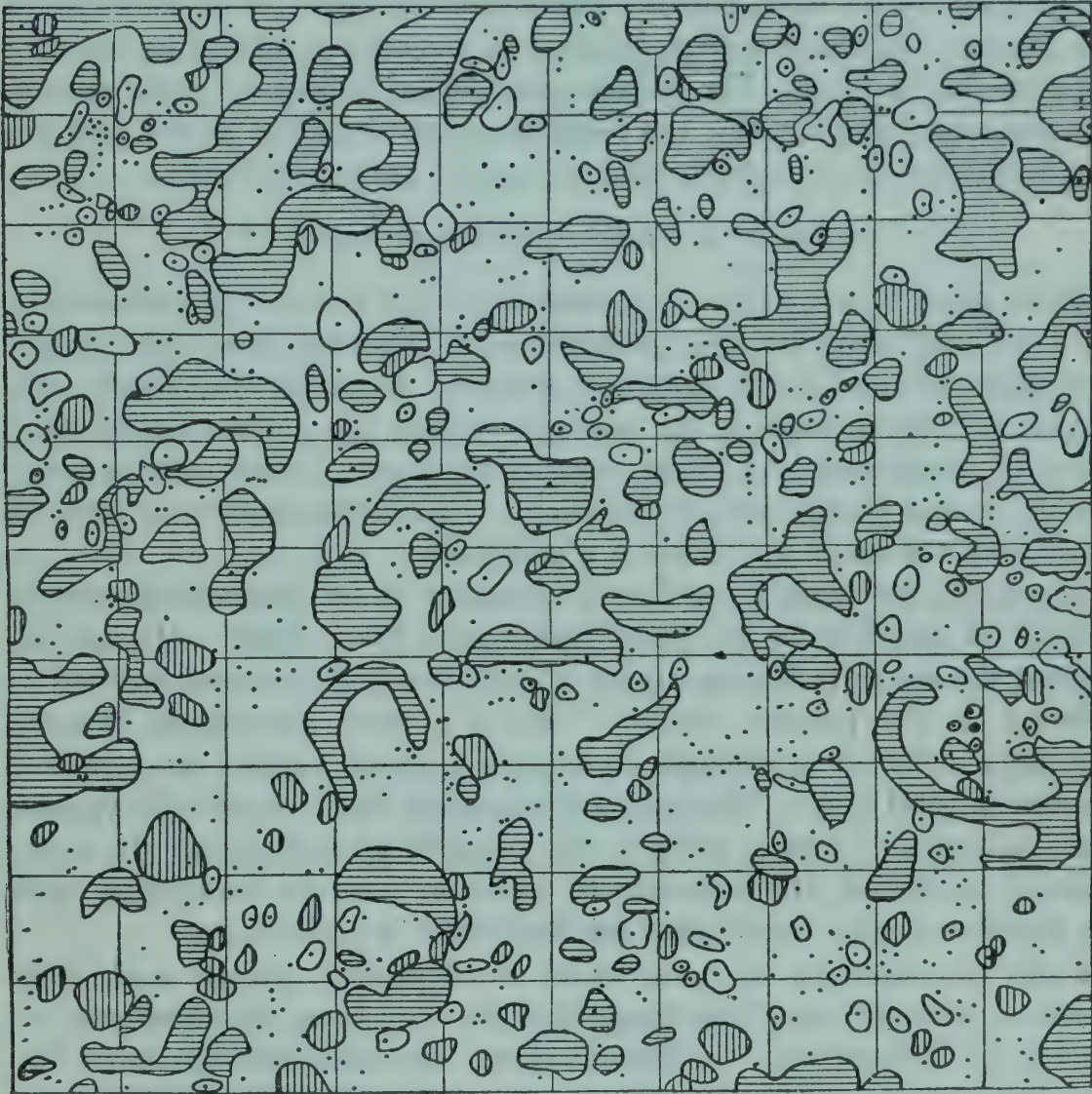


FIG. 2.—Meter quadrat in 30-acre pasture mapped in detail in 1915. Cross hatching represents *Bouteloua gracilis*; vertical hatching, *Stipa comata*. The presence of other species is indicated by dots and outlined areas.

the important point in relation to grazing systems. If grazing has been severe, the basal cover is likely to be changed rapidly, but under normal conditions it should change gradually. This is especially true in such regions as Mandan, where most of the vegetation is made up of perennial species. Sampson (5) says in regard to increase of ground cover:

The increase in actual stand or ground covered was due almost entirely to the enlargement of the tufts, and text figures 5 and 6 show that even under season-long protection the bunch-grasses and other valuable plants do not increase rapidly by this means.

Since the carrying capacity of the range is largely dependent upon the density of the vegetation, it is obvious that this factor should be carefully determined. If density is determined on the basis of the foliage cover, even when this is possible, the carrying capacity is likely to be placed too high, because of favorable growth conditions or an accumulation of previous growth, and overgrazing will result. In normal seasons the amount of forage a given area of ground surface can produce is largely determined by its basal cover. Therefore, the basis for an estimate of the amount of ground surface covered by vegetation should be founded upon the basal cover. The foliage cover is the important consideration for immediate grazing, but the basal cover more nearly determines the future possibilities of a given area of land for grazing purposes.

AMOUNT OF BASAL COVER AT MANDAN

From quadrat maps drawn to show bare and covered ground surface the total basal cover has been determined. The maps show about 60 per cent vegetated and 40 per cent bare ground. From quadrat maps, such as that in figure 2, made in the various pastures, the percentages of basal cover of *Bouteloua* and *Stipa* were determined. These are approximately 20 and 10 per cent, respectively. These determinations were all made from the maps by means of a planimeter.

Shantz (7) has made a number of estimates on the amount of cover in a series of quadrats in the mesa region near Pikes Peak. He has expressed the amounts in percentages in each case. The same method is followed in the present studies. This is a most convenient system, especially when it is desired to express a given species in terms of amount of total cover. Sampson (5) expresses the "density of vegetation" in terms of tenths, using 10 as complete ground cover. In order to avoid confusion, the amounts of cover as used in connection with the Mandan grazing experiment are expressed in percentages.

From the amounts of basal cover of *Bouteloua gracilis* and *Stipa comata* it is readily seen how important they are from the standpoint of grazing in this section. Griffiths, Bidwell, and Goodrich (2) have discussed the value of these grasses for forage. From clipping experiments at Mandan in 1917, in connection with the grazing studies, the *Bouteloua* was found to have produced from 40 to 50 per cent and *Stipa* from 15 to 20 per cent of the total forage for the season. When the quadrats were clipped, the vegetation was separated into six parts, as follows: *Bouteloua gracilis*, *Stipa comata*, *Aristida longiseta*, other grasses, *Carex filifolia* and *C. heliophila*, and other plants. Columns are also reserved for the sum of *B. gracilis* and *S. comata* and for the total weight of all grasses and of all species. From these data it is possible to determine the relation of one species or group to another or to the total weight of all species. The various amounts were recorded in grams, weighed both green and air-dried. From these data it appears evident that the ground layer is the important one from the standpoint of grazing in this section.

The abundance of a given species often appears greater than is determined by actual counts per unit area. Pound and Clements (4) have fully discussed this point. From Plate 12 it would appear that *Psoralea argophylla* is the most abundant species. However, by a number of actual counts per unit area it was found to be fourth in abundance of plants other than grasses and sedges.

SUMMARY

(1) The data and conclusions presented in this paper have been obtained in connection with a grazing experiment at the Bureau of Plant Industry Field Station near Mandan, N. Dak. This experiment is designed to determine the carrying capacity of the native vegetation and the effects upon it of different intensities and methods of grazing.

(2) The vegetation is composed of a large number of species, only a few of which produce a considerable amount of the total forage. The dominating species are *Bouteloua gracilis* and *Stipa comata*.

(3) The density of the vegetation is determined by the thickness of plants upon the ground surface and not by the foliage growth. The term cover used in connection with density may mean basal cover or foliage cover. The former remains after the latter has been removed by close grazing or clipping.

(4) The total basal cover of all species in the Mandan region is approximately 60 per cent of the ground surface. *Bouteloua gracilis* has a basal cover of about 20 per cent and *Stipa comata* nearly 10 per cent of the ground surface.

(5) Clipping data of different day periods showed that *Bouteloua gracilis* had produced from 40 to 50 per cent and *Stipa comata* from 15 to 20 per cent of the total forage. The remainder was made up of a number of other species.

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PLATE 12

General view of native vegetation near Mandan, N. Dak., showing composition and density. The following species are evident in the photograph: *Psoralea argophylla*, *Echinacea angustifolia*, *Artemisia frigida*, *Bouteloua gracilis*, *Stipa comata*, *S. viridula*, and *Ratibida columnaris*.

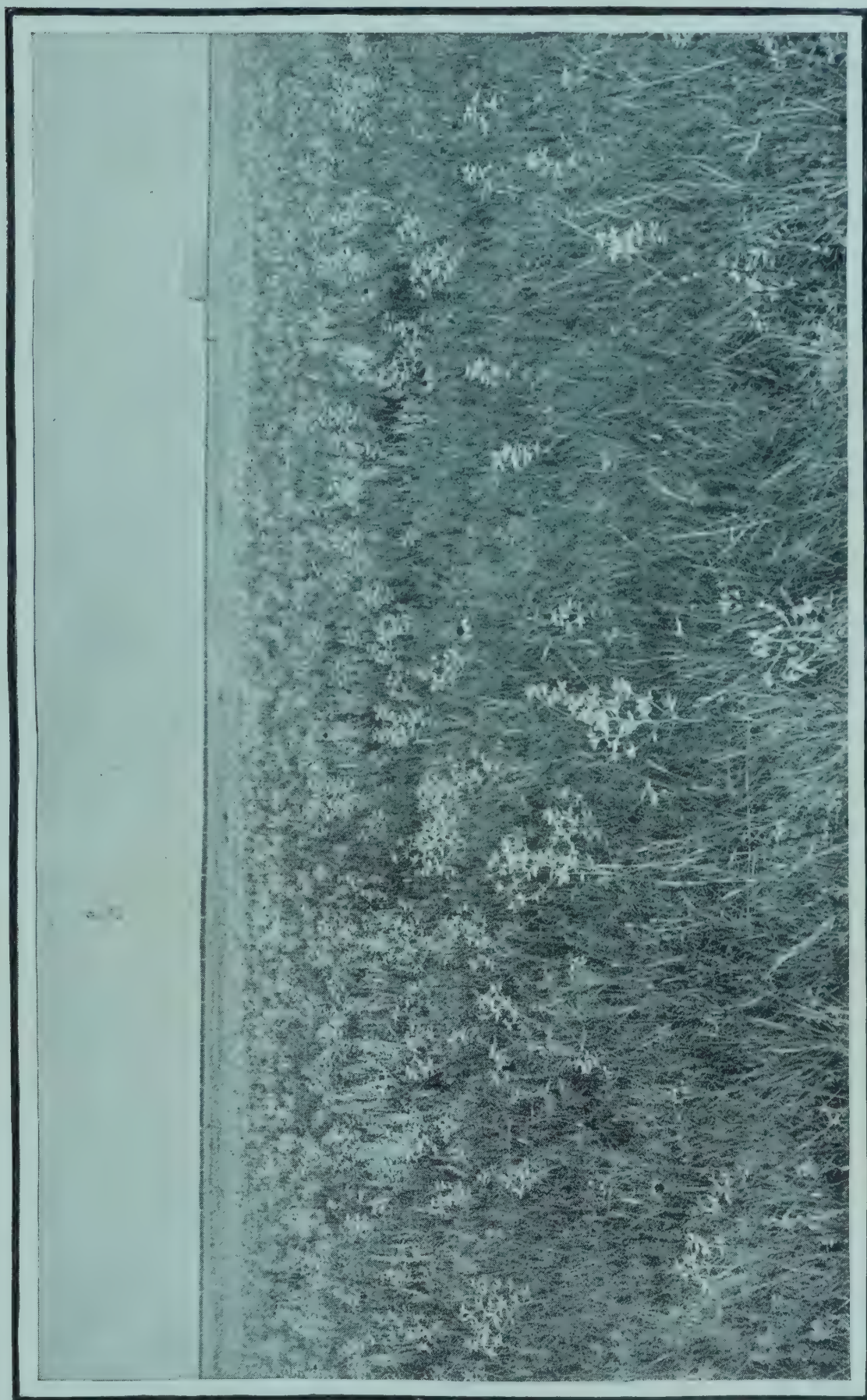




PLATE 13

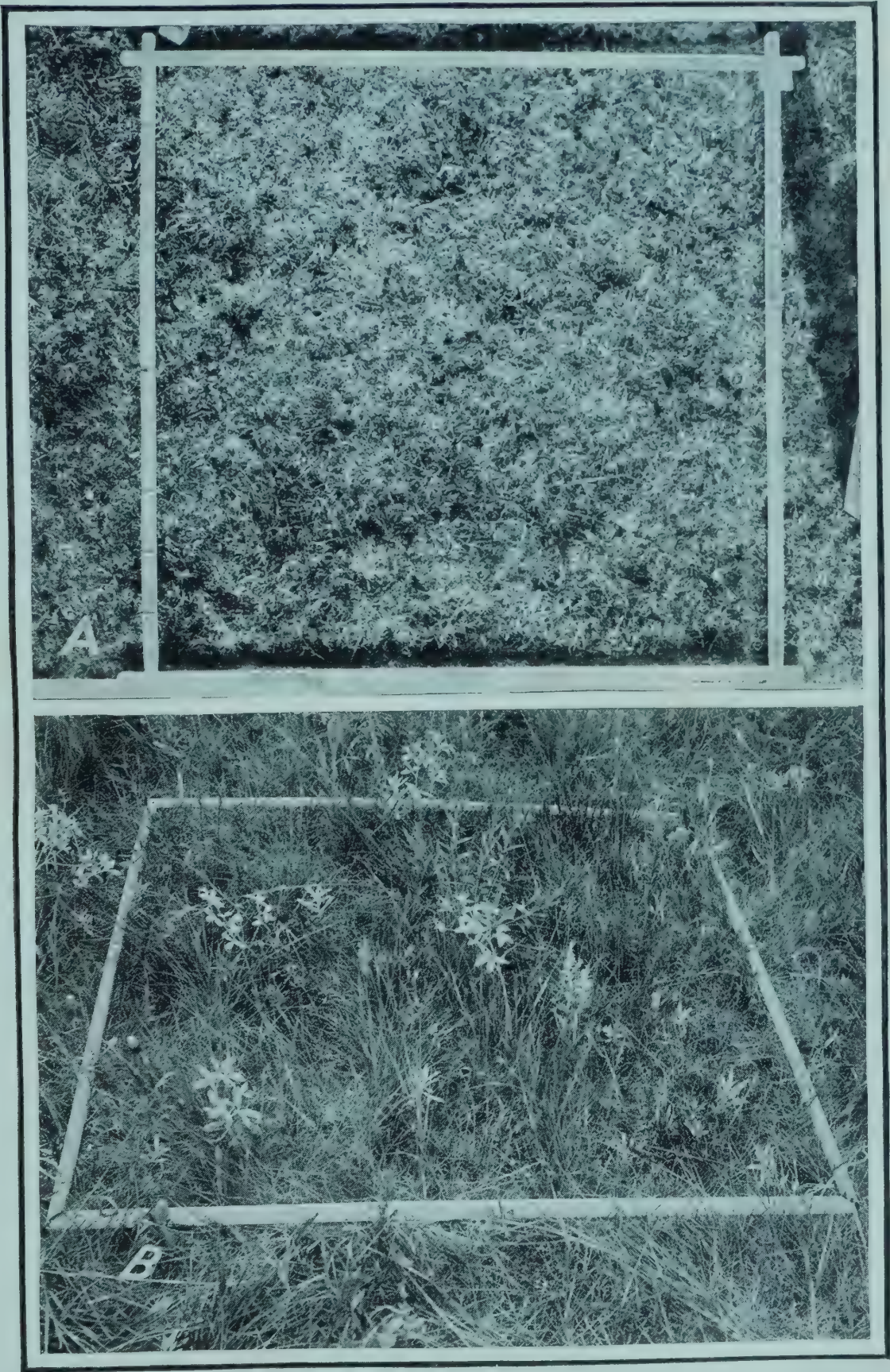
A.—View across area of *Andropogon furcatus*. This grass is closely grazed, as it is greatly relished by cattle. Mandan, N. Dak., Nov. 2, 1917.

B.—Close view of *Aristida longiseta* bunches. All other vegetation has been removed by cattle close to the bunches. Mandan, N. Dak., Nov. 2, 1917.

PLATE 14

A.—Close view, from above, of meter quadrat in 30-acre pasture. This is the same area shown in B but was taken in 1916 after the foliage cover had been removed by grazing. Only basal cover remains. Mandan, N. Dak., Oct. 10, 1916.

B.—Meter quadrat in 30-acre pasture. This shows the cover as it appeared before grazing. Mandan, N. Dak., July 28, 1915.



EFFECT OF REACTION OF SOLUTION ON GERMINATION OF SEEDS AND ON GROWTH OF SEEDLINGS

By ROBERT M. SALTER, *Soil Chemist*, and T. C. McILVAINE, *Assistant Agronomist*,
West Virginia Agricultural Experiment Station

INTRODUCTION

Recent investigations have emphasized the importance of the intensity factor of soil acidity. The growth of plants is more logically associated with hydrogen-ion concentration than with total acidity as measured by a soil's capacity to neutralize or absorb bases. However, other factors than the direct physiological influence of the hydrogen or hydroxyl ion upon the plant itself are undoubtedly operative in producing the effects attributed to soil reaction, these factors being either conditioned by the reaction or associated with it. Thus, indirect effects upon plant growth would be produced by: (1) The extent to which the soil's reaction is favorable for the development of soil organisms, more particularly those responsible for nitrogen transformation and nitrogen accumulation; (2) changes in the solubilities of soil constituents as affected by reaction, this applying not only to essential elements such as calcium, magnesium, potassium, and phosphorus but also to those having toxic properties, such as aluminium, manganese, and ferrous iron where increases in concentration would be expected with increase in acidity; and (3) changes produced in physical properties of soils attendant upon changes in reaction.

Although the mass of data on the relation of soil acidity to plant growth is already large, few well-defined attempts have been made to separate the individual factors concerned and study them under conditions permitting the control or elimination of other factors. The present investigation was undertaken with the aim of studying the direct physiological influence of reaction as measured by hydrogen-ion concentration upon plant growth. Solution culture was resorted to in order to control or eliminate other factors as far as possible.

EXPERIMENTAL METHODS

In the work herein reported, wheat, corn, soybean, and alfalfa seedlings were grown in a series of solution cultures having as far as possible a constant nutrient composition and osmotic concentration and varying in reaction from a hydrogen-ion concentration of approximately 1×10^{-2} to 1×10^{-8} or 2 P_H to 8 P_H .¹

¹ In this report the P_H values of Sorensen will be used to state the reaction of the solutions, the value P_H being the negative common logarithm of the actual numerical concentration of hydrogen ions. Thus a concentration of hydrogen ions of 1×10^{-5} would correspond to a P_H value of 5.

NUTRIENT COMPOSITION OF SOLUTIONS

The need for a basic nutrient culture of favorable physiological balance was recognized. The attempt was at first made to adjust Shive's solutions No. R₅C₂ and R₃C₃ (24),¹ which he found best suited to the growth of wheat seedlings, to the various reactions desired for the work by additions of the requisite amounts of an acid or base. However, because of the extensive precipitation of phosphates of calcium and magnesium in the more alkaline members of such series, these solutions were found unsuited to the work at hand.

Two series of solutions were eventually employed which varied somewhat in composition from Shive's best solutions. The maximum partial ionic concentrations in volume equivalents for the two solutions used are given in Table I, the composition of Shive's solutions being included for purposes of comparison.

TABLE I.—Maximum ionic concentrations of solutions
[Expressed as gram-equivalents per liter]

Kind of solution.	Na+.	K+.	½Ca++.	½Mg++.	NO ₃ -.	½SO ₄ -.	H ₂ PO ₄ -.	H ₃ C ₆ H ₅ O ₇ -.	Cl-.
Series A..	{ 0.0100 to .0200 }	{ 0.0360 }	0.0050	0.0050	0.0100	0.0050	0.0180	{ 0.0100 to .0000 }	{ 0.0050 }
Series B..	{ .0000 to .0360 }	{ .0180 }	.0050	.0050	.0100	.0130	.01800050
Shive's									
R ₅ C ₃0180	.0104	.0300	.0104	.0300	.0180
R ₃ C ₃0108	.0156	.0400	.0156	.0400	.0108

The salts, acids, and base used and their volume-molecular concentrations were as follows:

Series A.—Dipotassium phosphate (K₂HPO₄), 0.0180 m.; sodium nitrate (NaNO₃), 0.0100 m.; calcium chlorid (CaCl₂), 0.0025 m.; magnesium sulphate (MgSO₄), 0.0025 m.; sodium hydroxid (NaOH), 0.0000 to 0.0100 m.; and citric acid (H₃C₆H₅O₇), 0.0100 to 0.0000 m.

Series B.—Potassium sulphate (K₂SO₄), 0.0040 m.; potassium nitrate (KNO₃), 0.0100 m.; CaCl₂, 0.0025 m.; MgSO₄, 0.0025 m., phosphoric acid (H₃PO₄), 0.0180 m., sodium hydroxid (NaOH), 0.0000 to 0.0360 m.

To each 500 cc. of culture solution there were added 5 drops of a ferric phosphate solution containing 0.25 gm. of FePO₄ per 100 cc.

VARIATION OF REACTION

The ideal method of adjusting the reaction in such a series of cultures

¹ Reference is made by number (*italic*) to "Literature cited," p. 93-95.

would be one which would permit a variation in unit steps over the desired range and at the same time produce solutions of sufficient stability to prevent small changes in the total amount of acid or base from seriously affecting the reaction. In other words, the solution should have a "buffer" nature. In the titration of strong acids with strong bases, a point is reached, as neutrality is approached, at which further additions of small increments of base produce very rapid decreases in the hydrogen-ion concentration. This corresponds to a rapid rise in the voltage curve obtained in the electrometric titration of such solutions. Any solution selected within this region of rapid change is unsuited to work requiring constancy of reaction, particularly when subject to possible small changes in total acidity. With acids and bases of low dissociation this difficulty is not so marked, changes of reaction being much less abrupt under similar conditions.¹ Such solutions are commonly said to possess a buffer nature and are well adapted to work similar to that herein reported.

In series A the reaction was varied by adding $\text{H}_3\text{C}_6\text{H}_5\text{O}_7$ and NaOH to the successive cultures in amounts equivalent to the following volume-molecular concentrations:

Culture No.	$\text{H}_3\text{C}_6\text{H}_5\text{O}_7$.	NaOH .
	M.	M.
1.....	0. 0100	0. 0000
2.....	. 0080	. 0020
3.....	. 0060	. 0040
4.....	. 0040	. 0060
5.....	. 0030	. 0070
6.....	. 0020	. 0080
7.....	. 0000	. 0100

The reaction curve as determined by the hydrogen electrode for this series is shown in figure 1, A.² It will be noted that this solution possesses sufficient buffer action to prevent any rapid changes in reaction with change in total content of acid and base.

¹ For a more complete discussion of this subject see Hillebrand (10).

² The measurements of hydrogen-ion concentration were made by means of the gas chain and hydrogen electrode, using the potentiometer system and measuring electromotive force to 0.0001 volt. For electro-metric titrations a special cell equipped with mechanical stirring device was designed.

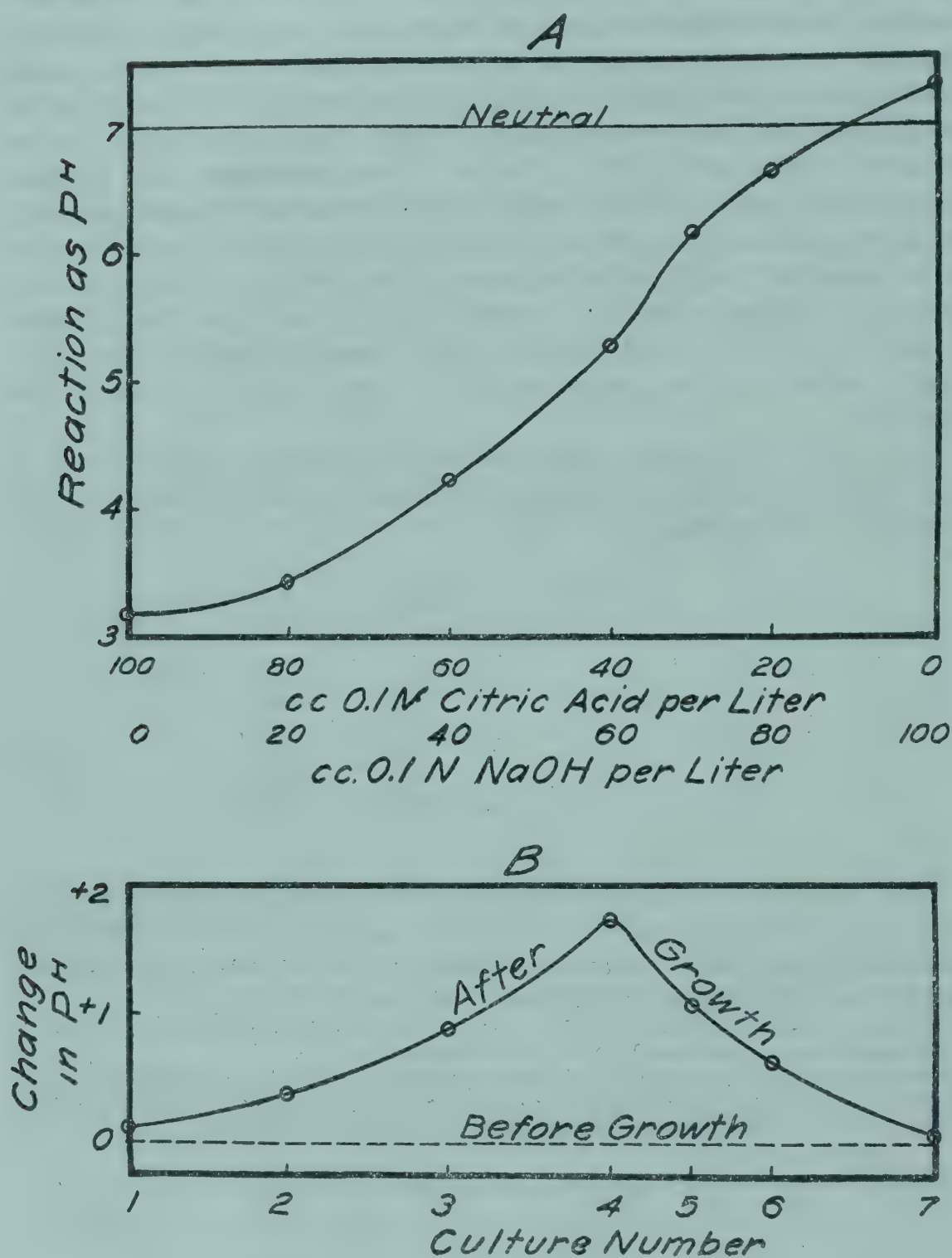


FIG. 1.—A, graph showing the relation of reaction to the contents of $\text{H}_3\text{C}_6\text{H}_5\text{O}_7$ and NaOH employed in the cultures of series A; B, graph showing the change of reaction found after 4 days' growth of wheat seedlings in series A.

In series B the reaction was varied by adding to all cultures sufficient H_3PO_4 to make the solution 0.0180 molecular and then NaOH in the following volume-molecular concentrations:

Culture No.	NaOH.
	M.
1.....	0. 0000
2.....	. 0144
3.....	. 0174
4.....	. 0181
5.....	. 0198
6.....	. 0288
7.....	. 0360

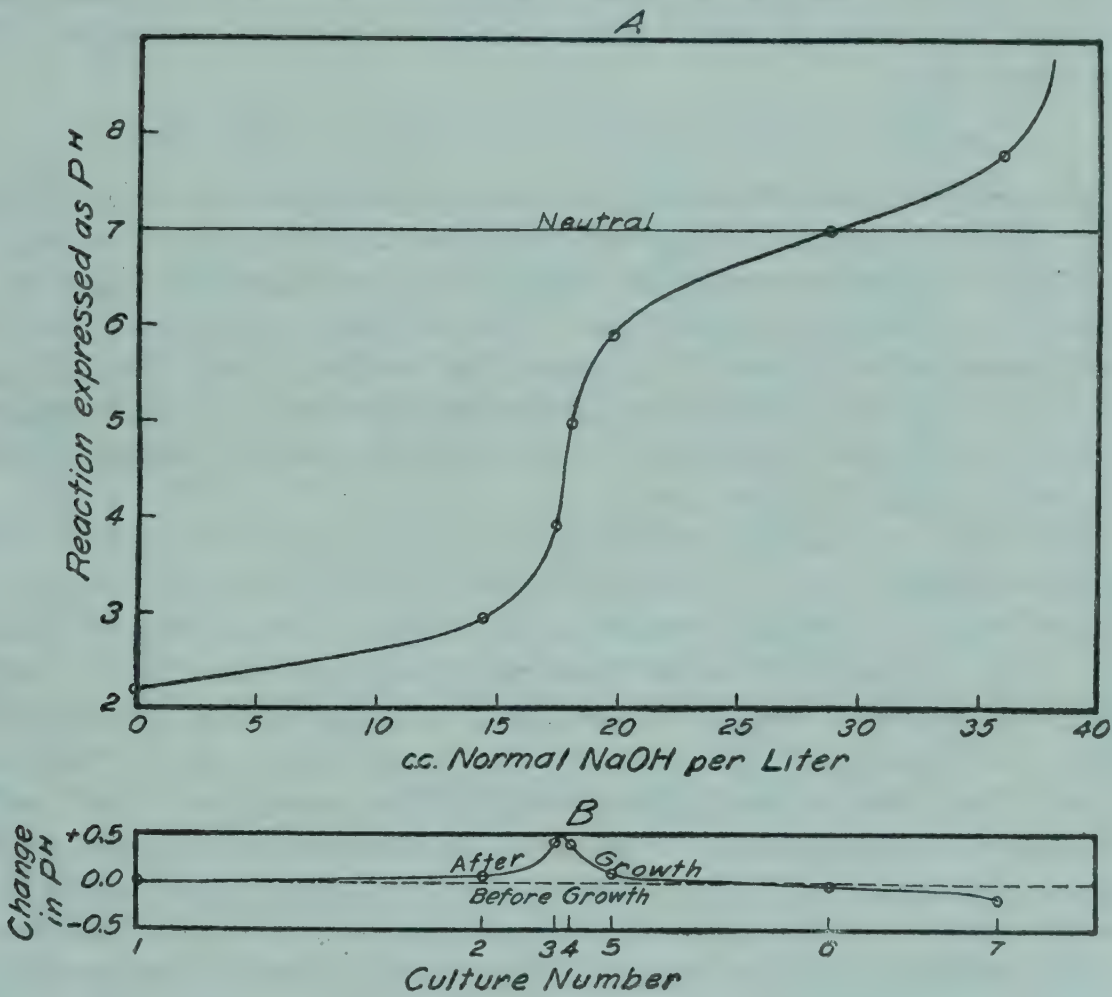


FIG. 2.—A, graph showing the change in reaction obtained by electrometric titration in series B; B, graph showing the change of reaction found after 4 days' growth of wheat seedlings in cultures of series B.

The electrometric titration curve for this solution (fig. 2, A) was used as a basis for determining the amounts of NaOH necessary to produce a series of seven cultures ranging from about 2 P_H to about 8 P_H and increasing in approximately equal steps of 1 P_H . The curve shows a rather abrupt rise at a point representing the complete neutralization of one hydrogen ion of the H_3PO_4 molecule. As will be shown later, the solutions chosen upon the steep part of the curve were less stable in reaction than those chosen upon the more nearly horizontal parts of the curve.

OSMOTIC CONCENTRATION

The osmotic concentrations of the solutions were not determined, because the data on the electrolytic dissociation of the component acids and salts under the variety of reactions used is not available and the authors did not have access to the necessary apparatus for making cryoscopic determinations. However, the relatively small change in total volume-molecular concentration within either series would indicate that little, if any, difference in growth within a given series should probably be attributed to the osmotic factor.

WATER EMPLOYED

All cultures were made from distilled water which had been rendered nontoxic by treating with carbon black as first recommended by Livingston (14).

TECHNIC OF GERMINATION AND GROWTH OF SEEDLINGS

The seeds of wheat, soybeans, and corn were germinated by supporting them upon a paraffined wire gauze which was floated by means of corks so that it was just even with the surface of nontoxic distilled water contained in a porcelain enameled pan. The seedlings were transferred to the various cultures when the plumules had attained a length of from 4 to 5 cm. The alfalfa seeds were germinated upon pads of filter paper in Petri dishes and transferred to the cultures after the seedling had attained a length of about 4 cm.

The wheat and alfalfa seedlings were grown in Non-Sol and Pyrex beakers holding 250 cc. of culture solution and were supported upon perforated caps of paraffined cheesecloth according to the method of Haas (7). The corn and soybean seedlings were grown in 8-ounce jars of flint glass and supported with corks according to the method of Tottingham (26). All beakers and jars were covered with black paper to exclude light. The solutions were renewed on all cultures every fourth day, and the glassware was thoroughly cleansed and sterilized before being used again. The reactions of the solutions used for growing wheat seedlings in both series were determined both before and after the 4-day periods. It was found that the successive solutions made up for a given reaction varied from each other by negligible amounts, so the solutions used for the growth of soybean, corn, and alfalfa seedlings were tested only at irregular intervals.

EXPERIMENTAL DATA AND DISCUSSION OF RESULTS

SERIES A

Wheat seedlings were grown for a period of 16 days in solutions having the composition given for series A. Growth was determined by taking the green weight of roots and tops, exclusive of seeds. Twelve seedlings were grown in each culture, and all seven cultures of the series were duplicated. The duplicate cultures agreed closely in all cases and are there-

fore not reported separately. The green weights obtained for tops, roots, and entire plants, exclusive of seeds, are given in Table II. The average reaction of each culture at the beginning and at the end of the 4-day periods and for the entire 16 days is also included in the table. The relative total green weights, based upon the highest, taken as 100, are shown in figure 3 plotted against the average P_H of the solutions, and the appearance of the seedlings at time of harvesting is shown in Plate 15, A, B.

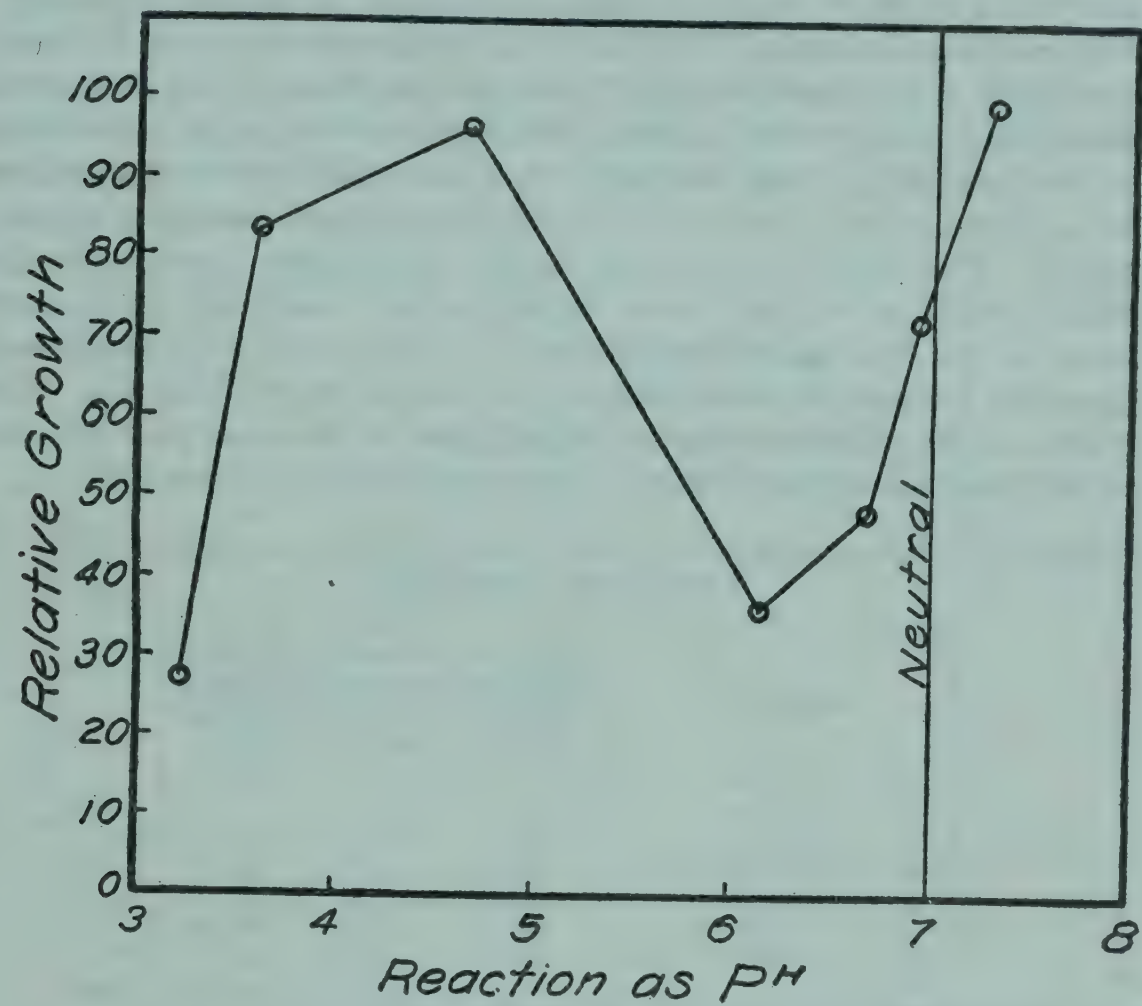


FIG. 3.—Graph showing the relation of growth of wheat seedlings to reaction in series A.

TABLE II.—Average reaction of cultures in series A and green weights of plants grown for period of 16 days

Culture No.	Average reaction of culture.			Green weight of 10 plants.		
	Before growth.	After growth.	Entire period.	Tops.	Roots.	Entire seedling.
	P_H .	P_H .	P_H .	Gm.	Gm.	Gm.
1.....	3. 18	3. 29	3. 23	0. 410	0. 049	0. 459
2.....	3. 44	3. 80	3. 62	1. 345	. 079	1. 425
3.....	4. 22	5. 10	4. 67	1. 440	. 204	1. 644
4.....	5. 26	7. 02	6. 14	. 570	. 049	. 619
5.....	6. 15	7. 21	6. 68	. 737	. 091	. 828
6.....	6. 61	7. 24	6. 92	1. 087	. 171	1. 258
7.....	7. 28	7. 34	7. 31	1. 375	. 331	1. 706

A brief consideration of the results obtained in this series shows them to be abnormal, since one would scarcely expect the decided drop in growth in cultures 4, 5, and 6 if reaction were the only factor concerned. The fact that there developed a decided opalescent or colloidal appearance in these cultures in about 24 hours after their renewal, together with the fact that there was a large decrease in acidity during the 4 days' growth of seedlings indicated that they were infected with some bacterial organism which evidently used the citric acid present as a source of energy. Microscopic examination of these solutions showed this to be the case, and it was at once surmised that the depressant effect of these solutions upon the growth of wheat seedlings was probably due to the assimilation of the nitrates by these bacteria. This hypothesis was substantiated by a determination of nitrates in all seven cultures at the end of a 4-day period. The relative total green weights of seedlings, based upon the highest taken as 100, the relative nitrate content, based upon the highest taken as 100, and the relative decrease in acidity of the solutions, based upon the greatest decrease taken as 100, are shown in Table III. The relation of the change in reaction taking place in the 4-day period to the original reaction of the solution is shown graphically in figure 1,B.

TABLE III.—Comparative total green weights, nitrate content, and acidity of cultures of series A at end of 4-day period

Solution No.	Relative yield (green weights of whole plants).	Relative amount of nitrates at end of 4-day period.	Relative decrease in acidity (increase in P _H).
	Gm.	Gm.	
1.....	26.9	84.0	6.2
2.....	83.5	92.8	22.2
3.....	96.4	78.0	50.0
4.....	36.3	6.0	100.00
5.....	48.5	7.8	60.3
6.....	73.7	24.0	35.8
7.....	100.0	100.0	3.4

The data show that depression in growth in cultures 4, 5, and 6 is associated with low amounts of nitrates left in solution and with large decrease in acidity. It seems safe, therefore, to conclude that the bacteria present were responsible for the abnormal effects obtained in this series. It should be noted that although there was more citric acid available to the bacteria in culture No. 3 than in No. 4, there was actually much smaller assimilation of nitrates in the former culture, while the wheat growth in No. 3 was almost equal to that in the best member of the series. Apparently the acidity of this culture has suppressed the growth of the nitrate-assimilating bacteria but has not had a correspondingly unfavorable effect on the growth of wheat seedlings. Since there was little difference in the amounts of nitrates present in cultures 1, 2, and 3 it seems

probable that the depression in growth found in cultures 1 and 2 was due to the physiological effect of their reaction upon the wheat seedlings.

The results obtained from this series do not give accurate data concerning the effect of reaction upon the growth of wheat seedlings over the entire range investigated. It seemed well, however, to include them in this report on account of their bearing upon a large amount of investigative work showing the ability of bacteria and fungi to compete with higher plants for inorganic nitrogen if supplied with a proper source of energy and carbon in the form of organic matter. This power of microorganisms has been demonstrated by numerous investigators under both solution and soil-culture methods. For a more complete discussion and an extensive bibliography on this subject the reader is referred to the publication of Doryland (4).

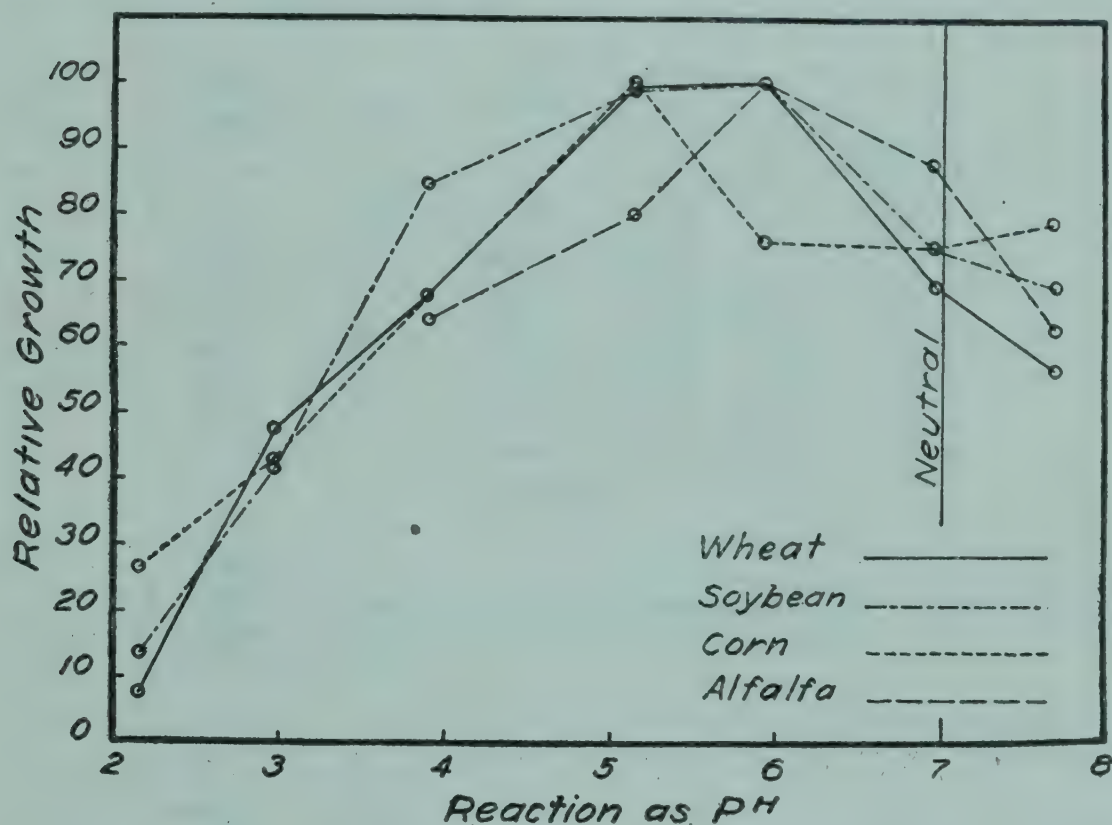


FIG. 4.—Graphs showing the relation of growth of wheat, soybean, corn, and alfalfa seedlings to reaction in series B.

SERIES B

On account of the difficulties arising from bacterial infection when citric acid was employed in the cultures, further work was confined to solutions having the composition given for series B. Wheat, soybeans, corn, and alfalfa seedlings were grown, all cultures being duplicated in the wheat, corn, and alfalfa series and quadruplicated in the soybean series. The numbers of seedlings grown in each culture were as follows: wheat, 12; soybean, 6; corn, 4; alfalfa, 20. The following periods of

growth were maintained: wheat, 18 days; soybeans, 16 days; corn, 8 days; alfalfa, 20 days. In Table IV are given the green weights of seedlings at time of harvesting and the average reaction of each culture as shown by the determinations of hydrogen-ion concentration made at the beginning and end of the 4-day periods on the cultures of the wheat series only. In figure 4 the relative total green weights, based upon the highest weight taken as 100 in each instance, are shown plotted against the average P_H of the cultures. Plate 15, C, shows the appearance of the wheat plants at the time of harvesting.

TABLE IV.—Average reactions of cultures of series B and green weights of seedlings at time of harvesting

WHEAT

Culture No.	Reaction.	Green weight of 10 plants.		
		Tops.	Roots.	Entire plants exclusive of seeds.
	P_H .	Gm.	Gm.	Gm.
1.	2. 17	^a 0. 230	^a 0. 067	^a 0. 297
2.	2. 96	1. 730	. 143	1. 873
3.	4. 11	2. 548	. 149	2. 697
4.	5. 16	3. 581	. 372	3. 953
5.	5. 94	3. 620	. 356	3. 976
6.	6. 97	2. 421	. 324	2. 745
7.	7. 71	2. 103	. 141	2. 244

SOYBEANS

Culture No.	Reaction. ^b	Green weight of 10 plants (entire).
	P_H .	Gm.
1.	2. 17	^a 4. 93
2.	2. 96	7. 62
3.	4. 11	15. 76
4.	5. 16	18. 54
5.	5. 94	18. 74
6.	6. 97	14. 13
7.	7. 71	12. 87

^a Seedlings dead at time of harvesting.

^b Because of the uniformity of reaction of successive cultures made up to to represent a given reaction and the relatively small changes in reaction produced by growth of seedlings it is assumed that the average reactions found in the wheat series apply to the cultures of the soybean, corn, and alfalfa series. Occasional determinations on cultures of the latter series showed this to be true.

TABLE IV.—Average reactions of cultures of series B and green weights of seedlings at time of harvesting—Continued

CORN		
Culture No.	Reaction. ^a	Green weight of 10 plants, exclusive of seeds.
	<i>P_H</i> .	<i>Gm.</i>
1.....	2.17	^b 3.53
2.....	2.96	5.66
3.....	4.11	8.98
4.....	5.16	13.30
5.....	5.94	10.14
6.....	6.97	10.03
7.....	7.71	10.47

ALFALFA		
Culture No.	Reaction. ^a	Green weight of 10 plants (entire).
	<i>P_H</i> .	<i>Gm.</i>
1.....	2.17	(<i>b</i>)
2.....	2.96	(<i>b</i>)
3.....	4.11	0.317
4.....	5.16	.397
5.....	5.94	.496
6.....	6.97	.435
7.....	7.71	.310

^a Because of the uniformity of reaction of successive cultures made up to represent a given reaction and the relatively small changes in reaction produced by growth of seedlings, it is assumed that the average reactions found in the wheat series apply to the cultures of the soybean, corn, and alfalfa series. Occasional determinations on cultures of the latter series showed this to be true.

^b Seedlings dead at time of harvesting.

Before discussing the foregoing data mention should be made of the fact that while in practically all cases duplicate cultures agreed closely, there was occasionally considerable variation between the individual plants in a single culture of soybeans and corn, while in alfalfa there was considerable mortality among the plants in all cultures of the series. For this reason in drawing conclusions from the foregoing data the authors prefer to consider the work with soybeans, corn, and alfalfa as somewhat preliminary in nature. This does not apply to the wheat series, where no significant variations were found between the plants in the cultures representing a given reaction.

The effects of acids and alkalis upon seedlings grown in solution culture have been quite extensively investigated by Kahlenberg and True (13), Heald (9), Cameron and Breazeale (2), Hartwell and Pember (8), Breazeale and LeClerc (1), Dachnowski (3), Miyake (18), Gedroitz (6), Loew (15), and Hoagland (11). A complete review of the reports

covering the work of these investigators is not deemed necessary in this paper, however, since with the exception of that of Hoagland, none of the foregoing researches are comparable with that herein reported, for the reason that actual measurements of hydrogen-ion or hydroxyl-ion concentrations were not made, total titratable acidity or basicity being taken as a measure of the reaction. This leads to erroneous conclusions where substances possessing a buffer nature, such as phosphates, are present in solution. On the other hand, results obtained from the use of solutions of single acids or bases are probably abnormal, since they lack the antagonistic effects noted in more complete nutrient cultures and probably operative under soil conditions. Furthermore, solutions of single strong acids or bases of the concentrations ordinarily employed in such work are extremely unstable and liable to large changes in reaction. This is particularly true in alkaline solutions where absorption of atmospheric carbon dioxide is not prevented. With organic acids there is also the possibility of change in reaction due to bacterial infection similar to that noted under series A of the present study.

Hoagland (11) investigated the effect of reaction on the growth of barley seedlings grown in partial nutrient solutions of like osmotic concentration, in which the reaction was varied by the use of the various potassium phosphates. Reaction was determined by use of the hydrogen electrode. He found a hydrogen-ion concentration of 0.7×10^{-5} ($5.15 P_H$) to be favorable to growth, while a concentration of 0.3×10^{-5} ($3.50 P_H$) was very toxic. A concentration of hydroxyl ions greater than 1.8×10^{-6} ($8.25 P_H$) was found to be distinctly injurious, and when exceeding 2.5×10^{-5} ($9.40 P_H$) extremely toxic. It is unfortunate that no solution of reaction between $3.50 P_H$ and $5.15 P_H$ was employed in this work, since the former reaction was extremely toxic and the latter favorable to growth. This is particularly true, since it has been shown in the author's laboratory that this range of reaction represents a variation from a small to an unusually high total acidity (lime requirement) in soils.

In series B of the present study, a reaction of $5.94 P_H$ gave maximum growth of wheat and soybeans, and in both cases a reaction of $5.16 P_H$ was but slightly less favorable. With corn seedlings maximum growth occurred at a reaction of $5.16 P_H$, while a reaction of $5.94 P_H$ was considerably less favorable. Maximum growth of alfalfa occurred in the culture having a reaction of $5.94 P_H$, while a reaction of $5.16 P_H$ considerably depressed the growth. A reaction of $4.11 P_H$ was somewhat less favorable to soybeans and distinctly less so to corn, wheat, and alfalfa than a reaction of $5.16 P_H$. A reaction of $2.96 P_H$ resulted in the death of all alfalfa plants in the culture, and while there was some growth of wheat, soybeans, and corn, at the time of harvesting the leaves of all plants had begun to die at the tips. The roots of these plants produced no lateral growth at this reaction and at time of harvesting had turned

brown in color and supported vigorous growths of mold. It seems probable, therefore, that all plants would have died in these cultures and that 2.96 P_H is really below the critical reaction for all crops studied. A reaction of 2.17 P_H killed all seedlings a few days after transplanting, and while in Table IV weights are included for the seedlings from cultures having this reaction, these represent the weights of the dead seedlings at time of harvesting. Abundant growth of molds occurred upon the roots of all plants in cultures of this reaction. A reaction of approximate neutrality, 6.97 P_H , was found less favorable to the growth of seedlings of all four crops than a slightly acid reaction, while a reaction of 7.71 P_H still further depressed the growth of all crops excepting corn, where a slight increase was observed, the latter probably falling within experimental error. A study of the growth curves (fig. 4), shows that the optimum reaction for alfalfa was apparently higher than for the other crops studied. While maximum growth occurred at 5.94 P_H , a reaction of 6.97 P_H had a less injurious effect and a reaction of 5.16 P_H a more injurious effect than was found with wheat, soybeans, and corn. This agrees with the relative adaptation to soil reaction of the several crops commonly observed in field practice. In this connection it is interesting to note that Fred and Davenport (5) have recently shown that the critical reaction for the bacterium *Rhizobium leguminosarum*, symbiotically associated with alfalfa, is 4.9 P_H , while that for the corresponding organism associated with the soybean is 3.3 P_H .

CHANGE OF REACTION INCIDENT TO GROWTH

As previously noted, determinations of reaction by means of the hydrogen electrode were made upon the cultures of the wheat series at the beginning and end of each 4-day period—that is, before and after renewing the solution on each culture. The average reaction at the beginning and at the end of the 4-day periods for wheat in series B and the changes observed in reaction are given in Table V. The relation between the change of reaction and the position of a given culture with respect to the electrometric titration curve is brought out by a comparison of the curves shown in figure 2, A, and figure 2, B.

TABLE V.—*Change in reaction during 4-day periods*

Culture No.	Reaction before growth.	Reaction after growth.	Change in reaction.
	P_H .	P_H .	P_H .
1.....	2.17	2.17	0.00
2.....	2.94	2.98	+ .04
3.....	3.90	4.31	+ .41
4.....	4.95	5.36	+ .41
5.....	5.90	5.98	+ .08
6.....	6.99	6.95	- .04
7.....	7.79	7.62	- .17

It will be noted that the actual numerical value of the change in reaction is closely related to the stability of a given culture as indicated by the slope of the electrometric titration curve at the point representing the composition of the solution. There appears, however, to be a general tendency for the more acid cultures of the series to become slightly less acid while the more alkaline members tend to become slightly less alkaline. The conditions were not such as to permit accurate determination of the change in total titrable acidity or basicity produced by growth. However, if the points on the electrometric titration curve (fig. 2, A), corresponding to the reaction of each culture before and after growth of seedlings, are projected upon the horizontal axis representing quantity of total alkali added, it is found that in cultures 2 to 7, inclusive, there were no large differences in the quantitative value of the change in reaction—that is, a change in reaction of 0.41 P_H in culture 3 or 4 does not necessarily correspond to a greater change in total acidity than a change of 0.08 P_H in culture 5. The exact cause of the change in reaction, whether due to root excretions, to selective ionic absorption, or to other factors, was not determined. The results obtained agree with those of Pantanelli (20), who found a general tendency for plants grown in solution culture to regulate the reaction towards that most favorable to growth. The results agree also with the more recent work of Hoagland (11, 12), who found that barley grown in partial and complete nutrient cultures caused the reaction to approach that of approximate neutrality. On account of the difference in conditions the foregoing data are not necessarily contradictory to the results of Breazeale and LeClerc (1), who grew wheat seedlings in solutions of the single salts K_2SO_4 , potassium chlorid (KCl), and $NaNO_3$ and found a development of acidity in the potassium salts and of basicity in $NaNO_3$ apparently due to selective ionic absorption. However, in the more recent work of Hoagland (12), who grew barley plants in single salt solutions of KCl, K_2SO_4 , $MgSO_4$, potassium phosphate (K_3PO_4), ammonium chlorid (NH_4Cl), and $NaNO_3$, he found that—

in no case was a condition either of excessive OH ion or H ion concentration produced, although absorption had been active. The acid reaction when present was due to slightly dissociated acids, usually carbonic, or to acid salts in the case of NH_4Cl solution. Possibly in some cases organic acids were formed.

In this connection it should be mentioned that Haas (7) grew wheat seedlings in distilled water and found no change of reaction, measurements being made after carbon dioxide had been removed.

POSSIBLE INFLUENCE OF FACTORS OTHER THAN REACTION

While it seems probable that the variations observed in the growth of the seedlings under the range of reactions employed were the direct

result of the variation in reaction, yet it should be noted that certain other factors might have been operative to an undetermined extent.

Attention has already been called to the probable small variations in osmotic concentrations of the cultures within a given series. It seems doubtful whether such variations could have exerted any appreciable effect.

There was a variation in sodium content from an equivalent concentration of zero in culture 1 to 0.0360 in culture 7 of series B. It has recently been shown in the researches of Shive (25) that the substitution of an equivalent amount of sodium phosphate (NaH_2PO_4) for part of the potassium phosphate (KH_2PO_4) of a 3-salt nutrient culture produced considerable increases in the growth of soybean seedlings. In the present work, however, the greatest variations in growth were associated with the smallest changes in sodium content. Thus culture 2, to which had been added sodium as NaOH equivalent to 0.0144 m. was apparently below the critical reaction for all plants studied, while maximum growth of all plants was obtained in either culture No. 4 or No. 5 to which had been added NaOH equivalent to 0.0181 m. and 0.0198 m., respectively. It seems highly improbable that the variations in growth could have been to any appreciable extent induced by such small variations in the total sodium content.

The contents of calcium and magnesium employed in the cultural solutions were purposely kept low. (See Table I.) There was nevertheless a trace of precipitate of the phosphates of these metals formed in culture 6, which had a reaction of 6.97 P_H , and a somewhat more abundant precipitate in culture 7, which had a reaction of 7.71 P_H . To what extent the change in concentration thus produced might have influenced the results was not determined. Attention has previously been called to the possibility of similar changes in solubility of these elements at corresponding reactions under soil conditions.

In the work of Shive (25), previously mentioned, a toxicity of monobasic phosphates was shown toward soybeans grown in soil and in solution culture. While a general relation between the degree of injury sustained by the plants and the total acidity of the cultures was noted in this work, the fact that determinations of hydrogen-ion concentration were not made prevented accurate conclusions as to the actual part played by the acidity factor in the production of the injurious effects associated with the monophosphate group. The data obtained in the present study indicate that there was probably little effect of the H_2PO_4 group aside from that produced by the hydrogen ion formed in its dissociation. This is brought out by the fact that in culture 4 there was maximum growth of corn seedlings and very nearly maximum growth of wheat and soybean seedlings; whereas in the composition of this solution, H_3PO_4 equivalent to a concentration of 0.0180 m. and NaOH equivalent to a concentration of 0.0181 m. were employed—that is,

approximately enough alkali was used just to neutralize the first hydrogen ion of the H_3PO_4 molecule. The concentration of the monophosphate group was undoubtedly higher in this culture, therefore, than in any others of the series, since all the phosphorus present existed as the equivalent of monosodium phosphate. In the cultures below No. 4 an increasing part of the phosphorus exists as H_3PO_4 , while in the cultures above No. 4 an increasing amount exists as sodium phosphate (Na_2HPO_4).

THE EFFECT OF REACTION ON GERMINATION

The effects of acids and alkalies upon the germination of seeds have been studied by Promsy (22, 23), Micheels (16, 17), and Plate (21). The general conclusions can be drawn from these investigations that a slightly acid reaction is favorable to the germination of most seeds, while bases exert an injurious effect. The relation of germination to acidity varies considerably with seeds of different plants and with the acid used, organic acids being apparently more favorable than inorganic when used in equivalent amounts. This is probably due to their lower dissociation. Promsy found that the optimum concentration of acids ranged from 0.5 to 5 parts per thousand, depending upon the nature of the seed and the acid employed. Higher concentrations of acid inhibit or prevent germination. It is asserted that the effects of acids and bases on germination are a result of their favorable or unfavorable influence on the enzymic processes concerned.

The authors are not familiar with any work showing the effect of reaction on germination in which hydrogen-ion or hydroxyl-ion concentration is taken as a measure of the reaction, or with any work showing the relative sensitivity of germination and of the subsequent growth of the plant to reaction so determined. Breazeale and LeClerc (1), in explaining some of their results obtained in the growth of wheat seedlings in acid cultures, draw the conclusion that the depressant effect of acidity is greater during germination than in the subsequent growth of the plant. They explain this by assuming a high sensitivity of the enzymes concerned in germination, particularly the oxidases and peroxidases, to the acid condition. From a practical standpoint it would seem desirable to know to what extent the effects of soil acidity are due to its injurious influence on germination and to its effects on the subsequent growth of the crop. Numerous instances have come under the authors' observation in which seed planted in soils of high acidity apparently germinated normally but either ceased to grow or died after the plants had attained a small growth. This would indicate a condition opposite to the conclusion of Breazeale and LeClerc (1).

To investigate this point seeds of wheat, corn, soybeans, alfalfa, and red clover were germinated in solutions having the same nutrient composition and reaction as those used in the growth of seedlings in series B. The seeds were germinated upon pads of three ashless filters placed in Petri

dishes in which had been placed porcelain plates of such size as to prevent submersion of the filter paper in the solution except at the periphery of the dish. At the beginning of the experiment 20 cc. of the proper solution were added to each dish, allowed to stand 10 minutes, poured off, and replaced with 20 cc. of fresh solution. This was done in order to guard against change in concentration due to adsorption of solutes by the filter paper. The number of seeds germinated in each dish was as follows: alfalfa, 40; red clover, 50; corn, 10; soybeans, 10; wheat, 25. To avoid the effect of individual variation the dishes were triplicated in the test with corn and duplicated in the test with soybeans. The solution was renewed on all dishes every other day. The dishes were kept at room temperature for seven days, at which time a germination count

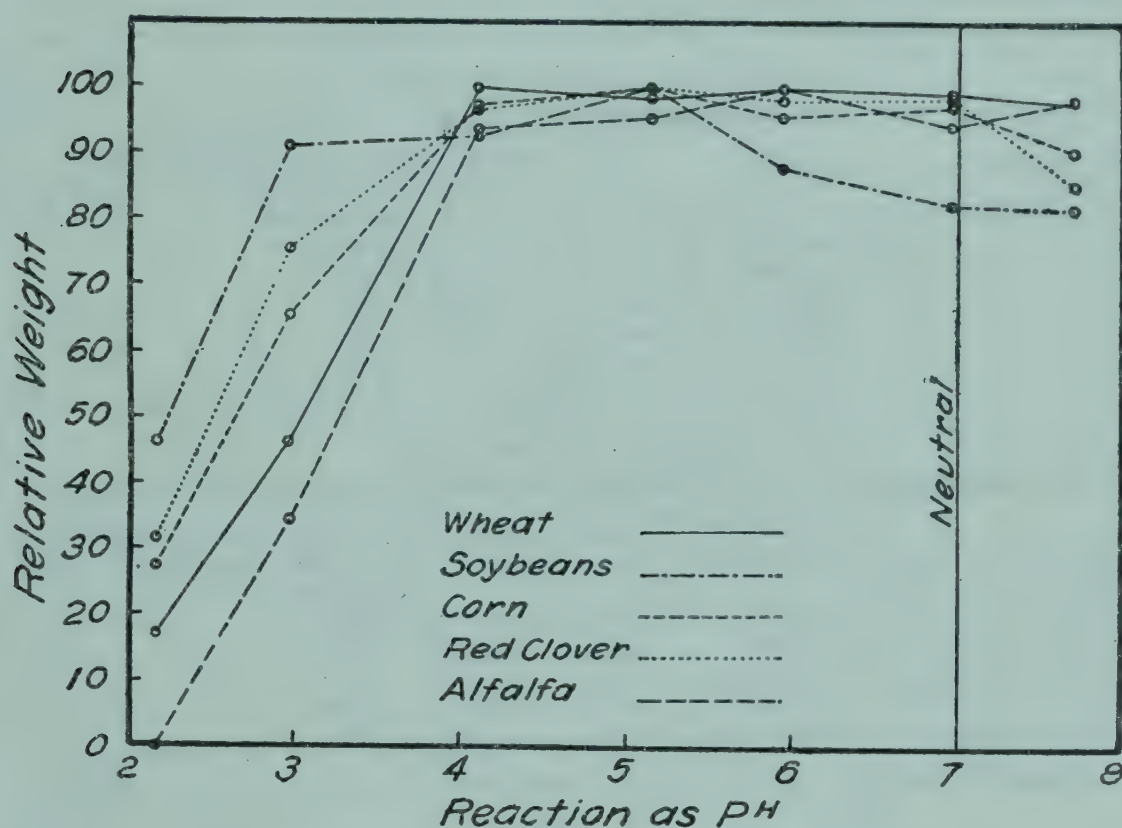


FIG. 5.—Graphs showing the relative weights of sprouts produced by seeds of wheat, corn, soybeans, alfalfa, and red clover in 7-day germination period at various reactions.

was taken and the green weight of the sprouts determined. The weight was taken for the entire seedling of the legumes, but the seeds were excluded in weighing the wheat and corn.

The number of seeds germinating in each culture and the average weights of the sprouts from 10 seeds are given in Table VI. The relative green weights of sprouts, based upon the largest weight taken as 100 in each instance, are shown plotted against the reaction of the cultures in figure 5.

TABLE VI.—Number of seeds germinating and average green weight of sprouts from 10 seeds

ALFALFA, 40 SEEDS TESTED

Dish No.	Reaction.	Number of seeds germinating.	Average weight of sprouts.	Notes.
	<i>P_H</i> .		<i>Gm.</i>	
1.....	2.17	0	Much mold, seeds swelled but no sprouts.
2.....	2.96	30	0.066	Some mold.
3.....	4.11	35	.181	No mold.
4.....	5.16	33	.184	Do.
5.....	5.94	33	.193	Do.
6.....	6.97	35	.182	Do.
7.....	7.71	27	.190	Do.

RED CLOVER, 50 SEEDS TESTED

1.....	2.17	5	0.048	Much mold, sprouts dead.
2.....	2.96	43	.115	Root tips brown and dead, some mold.
3.....	4.11	50	.148	No mold.
4.....	5.16	47	.153	Do.
5.....	5.94	45	.150	Do.
6.....	6.97	45	.151	Do.
7.....	7.71	47	.131	Do.

SOYBEANS, 20 SEEDS TESTED

1.....	2.17	4	1.665	Much mold, sprouts dead.
2.....	2.96	20	3.284	Root tips brown and dead, some mold.
3.....	4.11	18	3.350	No mold.
4.....	5.16	20	3.607	Do.
5.....	5.94	20	3.173	Do.
6.....	6.97	19	2.975	Do.
7.....	7.71	20	2.960	Do.

TABLE VI.—Number of seeds germinating and average green weight of sprouts from 10 seeds—Continued

CORN, 30 SEEDS TESTED				
Dish No.	Reaction.	Number seeds germinating.	Average green weight of sprouts.	Notes.
	P_H .		Gm.	
1.....	2. 17	26	0. 941	Small growth of mold, sprouts dead.
2.....	2. 96	29	2. 247	No mold.
3.....	4. 11	29	3. 357	Do.
4v.....	5. 16	30	3. 447	Do.
5.....	5. 94	30	3. 293	Do.
6.....	6. 97	29	3. 353	Do.
7.....	7. 71	29	3. 130	Do.

WHEAT, 25 SEEDS TESTED				
1.....	2. 17	16	0. 198	Much mold, sprouts dead.
2.....	2. 96	22	. 541	Some mold.
3.....	4. 11	25	1. 163	No mold.
4.....	5. 16	22	1. 143	Do.
5.....	5. 94	23	1. 164	Do.
6.....	6. 97	25	1. 156	Do.
7.....	7. 71	23	1. 141	Do.

While there is evidence of some abnormalities in the foregoing data, the authors believe the following conclusions are justified:

A reaction of 4.11 P_H did not exert a depressing effect on the germination of any of the seeds studied as measured by the germination count and the green weight of the sprouts at the end of the 7-day period. It will be recalled that the same reaction was found to depress the growth of seedlings of alfalfa, soybeans, corn, and wheat. Apparently the process of germination is not so susceptible to injury by acidity as is the subsequent process of growth with these plants.

A reaction of 2.96 P_H did not have any considerable effect upon the number of seeds germinating but considerably reduced the weight of the sprouts produced except with soybeans. In the latter case the roots had begun to turn brown in color and die at the tips at the end of the 7-day period. Some mold grew on the seeds in all dishes of this reaction.

Swelling of all seeds took place in dishes having a reaction of 2.17 P_H , and some small sprouts were produced from all seeds except those of alfalfa. All sprouts were apparently dead at the end of the 7-day period, and a severe growth of mold was present in all dishes of this reaction.

A reaction of 7.71 P_H decreased to a slight extent the weight of sprouts of all plants except alfalfa and wheat but did not appreciably lower the number of seeds germinating.

The optimum reaction for the germination of the seeds of the five plants studied is probably below 7.71 P_H and above 2.96 P_H .

GENERAL APPLICATION OF RESULTS TO FIELD PRACTICE

Most experiment stations recommend the use of such amounts of lime as will neutralize the total acidity present and maintain a soil at a neutral or slightly alkaline reaction. The results herein reported would indicate that if the direct physiological effect of excessive acidity upon plant growth were the only factor concerned, it would be more desirable to recommend such amounts of lime as would maintain the soil at a slightly acid reaction such as would be represented by a P_H value of 5 or 6. On the other hand, attention has been called to the necessity of further investigation of the other factors associated with acidity before this conclusion is warranted. Thus, a high optimum reaction (in P_H) for the development of the nitrogen-transforming organisms of a soil might counterbalance completely the advantages of a slightly acid reaction for the growth of the plant itself. The authors have investigations in progress, including solution, pot, and field plot studies, which it is hoped will give further evidence upon the part played by the other factors concerned.

SUMMARY

(1) A study has been made of the effects of reaction, as measured by hydrogen-ion concentration, upon the growth of the seedling of wheat, soybeans, corn, and alfalfa in solution culture and upon the germination of the seeds of wheat, soybeans, corn, alfalfa, and red clover, under conditions permitting the elimination or control of factors other than reaction.

(2) Citric acid was found unsuitable for adjusting the reaction of culture solutions for such work on account of bacterial infection which produced rapid changes in reaction and nitrate content. The nitrate-assimilating bacteria in this case were found more sensitive to acidity than were wheat seedlings.

(3) A satisfactory method of adjusting the reaction of the culture solutions was found to be the addition of a uniform amount of H_3PO_4 to all cultures and increasing amounts of NaOH to successive cultures.

(4) Maximum growth of seedlings of wheat, soybeans, and alfalfa occurred in cultures having a reaction of 5.94 P_H , while corn produced greatest growth in the cultures having a reaction of 5.16 P_H .

(5) A reaction of 5.16 P_H was approximately equal to 5.94 P_H for the growth of soybeans and wheat but decidedly less favorable for the growth of alfalfa.

(6) A reaction of 4.11 P_H was somewhat less favorable to soybeans and distinctly less favorable to corn, wheat, and alfalfa than a reaction of 5.16 P_H .

(7) A reaction of 2.96 P_H is probably below the critical reaction for all plants studied.

(8) A reaction of 2.16 P_H caused the death of the seedlings of all plants within a comparatively short time and was found to favor the growth of molds in the cultures.

(9) A reaction of approximate neutrality (6.97 P_H) was slightly less favorable to alfalfa and decidedly less so to wheat, corn, and soybeans than a slightly acid reaction.

(10) A reaction of 7.71 P_H produced further depression of growth beyond that observed at 6.97 P_H except in the case of corn seedlings.

(11) The hydroxyl ion was apparently more harmful than the hydrogen ion in equivalent concentrations.

(12) Measurements of reaction of solutions before and after the growth of wheat seedlings showed a general tendency for the plant to adjust the reaction toward a point slightly below neutrality.

(13) The actual value of the change of reaction produced by the growth of seedlings in a given culture was found to be a function of the stability of the solution as indicated by the slope of the electrometric titration curve at the point representing the composition of the solution.

(14) No indication was obtained of any harmful effect of the monophosphate group, H_2PO_4 , other than that produced by the hydrogen ion formed through its dissociation.

(15) Germination of the seed was found less sensitive to an acid reaction in wheat, corn, soybeans, and alfalfa than was the subsequent growth of the seedling.

(16) A reaction of 4.11 P_H did not exert a depressing effect on the germination of any of the seeds studied.

(17) A reaction of 2.96 P_H did not appreciably affect the number of seeds germinating but considerably reduced the weight and apparent vigor of the sprouts produced.

(18) A reaction of 2.16 P_H did not prevent the formation of sprouts except in alfalfa, but all sprouts produced were dead at the end of the 7-day germination period. This reaction induced the extensive growth of molds upon the seeds.

(19) The optimum reaction for the germination of the seeds of the five plants studied is probably below 7.71 P_H and above 2.96 P_H , a slightly acid reaction being found most favorable in all cases.

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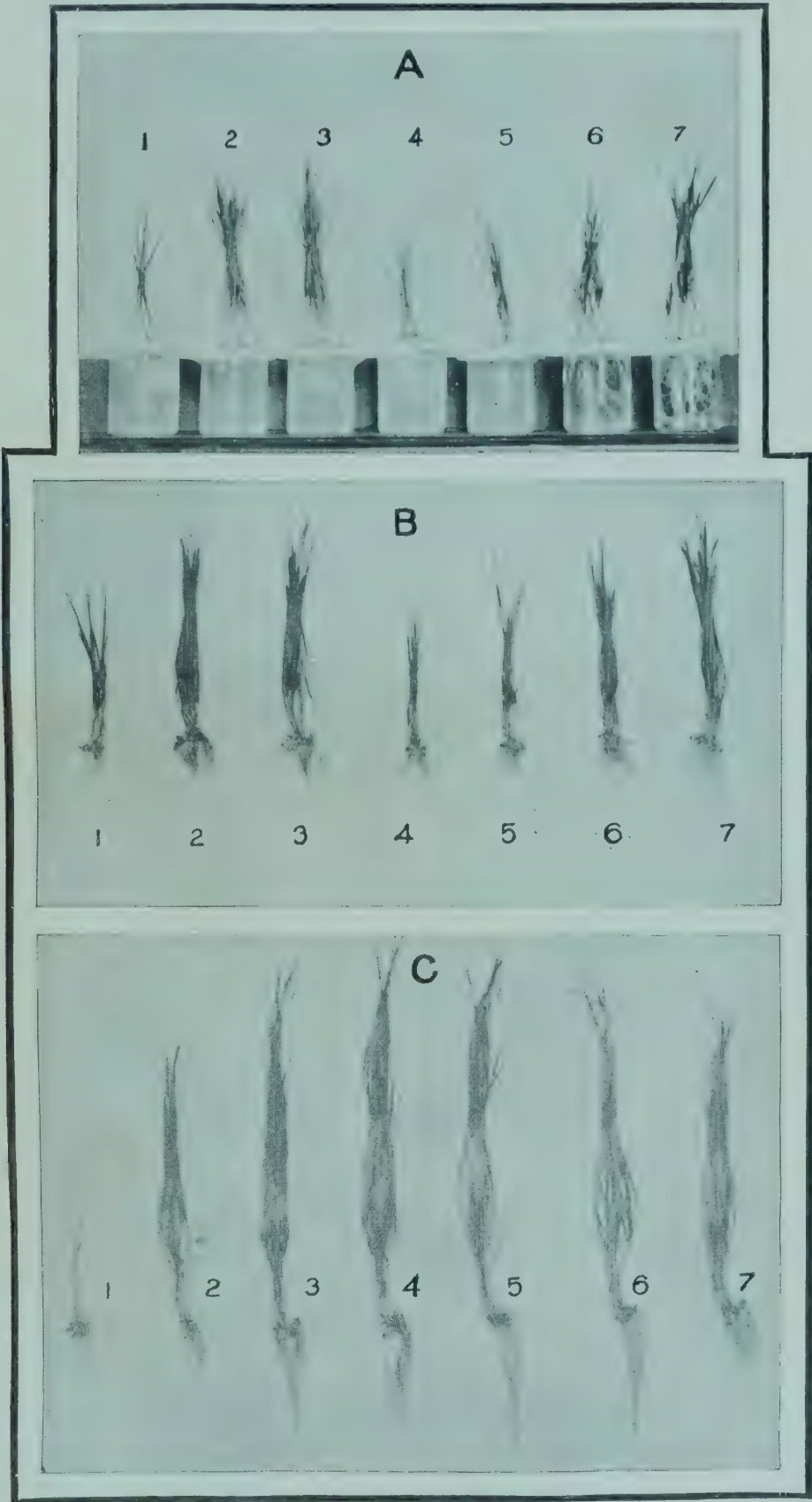
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PLATE 15

- A.—Method of growing wheat seedlings. (Paper covers removed from beakers.)
- B.—Appearance of wheat seedlings in series A at time of harvesting.
- C.—Appearance of wheat seedlings in series B at time of harvesting.



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PHILIPPINE DOWNY MILDEW OF MAIZE

By WILLIAM H. WESTON, JR.¹

*Pathologist in Charge of Downy Mildew Investigations, Office of Cereal Investigations,
Bureau of Plant Industry, United States Department of Agriculture*

During the past 20 years there have been reported from the Orient several downy mildew diseases of maize, sugar cane, and other economic grasses, caused by members of the genus *Sclerospora* of the *Peronosporaceae*. The most recently noted of these has been found in the Philippine Islands, where it causes very serious damage to maize, a crop which in area under cultivation is second only to rice. In 1916, a brief note by Prof. Baker (1)², of the College of Agriculture, first mentioned the occurrence and destructive power of the disease. In 1918, a short description of it with drawings of the causal fungus was published by Reinking (17). No further information concerning this dangerous disease has been published, but it is known that it occasions heavy and constant losses in the maize crop of this the richest of our oriental possessions and represents a grave potential menace to this extremely valuable crop of our own country.

The danger of the introduction of this disease to the cornfields of America was felt to be sufficiently grave to warrant a full investigation in the Philippines and elsewhere in the Orient. By such research it was expected to determine the distribution and life history of this organism and to devise methods of control. With these data in hand, the chances of promptly checking the disease were greatly increased should it gain a foothold in the United States at any time in the future. In the meantime, a quarantine was established against the importation of corn from the Orient.

It was the privilege of the writer to be detailed to this investigation, and since April, 1918, he has been at work on it in the Philippines.

The following paper deals with the general features of the disease and with the characteristics of the causal *Sclerospora* and its systematic position

¹ The writer wishes to express his thanks to Dean Baker, Prof. Reinking, Prof. Elayda, and others at the College of Agriculture of the University of the Philippines for so generously furnishing laboratory facilities, land, and other assistance; to Mr. S. Apostol, of the Philippine Bureau of Agriculture, for information on the distribution of the disease; and to Dr. E. D. Merrill, of the Bureau of Science, for many courtesies which have aided the progress of this investigation.

² Reference is made by number (italic) to "Literature cited," p. 121-122.

or relationship to other downy mildews destructive to cereal, forage, and sugar-cane crops in the Orient.

DISTRIBUTION

Broadly speaking, the disease is distributed throughout the Philippine Islands. Through the personal observation of the writer and through information given by Dr. Reinking, of the College of Agriculture, by his students, and by members of the Bureau of Agriculture, the disease is known to exist in the Cotobato Valley of the Island of Mindanao at the south, in the Islands of Cebu and Occidental Negros, and in the provinces of Batangas, Laguna, Rizal, Cavite, Bulacan, Tarlac, Pampanga, Nueva Ecija, Pangasinan, Ilocos Norte, la Union, and Isabela in Luzon at the north. In some of these localities the disease appears to have been present for more than 10 years, but as yet not enough is known to warrant a discussion of its probable origin.

DESTRUCTIVENESS

The disease is unusually destructive. It is impossible for one accustomed only to the comparatively light losses occasioned by the maize diseases of the United States corn belt to form any conception of the epidemic intensity of the attacks of this downy mildew under favorable conditions, or of the terrible destruction which it occasions (Pl. 16). Of the aggregate loss to the \$8,820,000 maize crop of the Philippines no estimate can be made, because farmers do not recognize the trouble as a disease but regard it as the result of excessive rain or other unfavorable conditions and accept it with fatalistic resignation. In Laguna and Batangas, however, where maize is a major crop and where the writer has studied the disease in the native fields, losses of 40 to 60 per cent are frequent, and in some cases as high as 82 per cent of infection has been counted. In the experimental and acclimatization plots at the College of Agriculture, where the growing of unacclimatized varieties and the constant presence of actively infecting plants combine to make the conditions especially favorable for infection, the losses ordinarily are high. In several beds of United States sweetcorn, planted during the rainy season, every plant was killed before producing any seed.

The severity of the disease in the individual corn plant varies with conditions from the extreme stunting and weakening of the plant resulting in death about one month after planting to the less virulent attacks in spite of which the plant shows a fair growth and ultimately produces a small, more or less poorly formed ear. Even in the few lightly affected cases the grain production is not nearly normal, and in most cases complete barrenness or premature destruction occurs, so the aggregate loss in the average field attacked by the mildew is large. In some localities corn growing has been abandoned for the culture of upland rice because of the ravages of the disease. Moreover, this loss can not be offset in part

by using diseased plants for fodder, for cattle appear to dislike the taste and will not eat the infected plants unless they are mixed with a liberal proportion of the healthy.

One of the most serious features of the attack by the downy mildew is that the infected plants are rendered susceptible to the attacks of a number of secondary parasitic organisms which contribute to the destruction of the weakened plants. In the rainy season there frequently occur destructive rots of the stem, ear, and shank, with which at least two species of *Pythium* and bacteria appear to be associated, while a species of *Helminthosporium*, which is only occasionally severe on healthy plants, is usually very destructive to plants weakened by downy mildew.

SYMPTOMS

The effect of the disease on the corn plant varies greatly with such conditions as the age of the plant when infected, the means by which infection takes place, the varietal nature and individual condition of the host, and the environmental conditions which accompany and follow infection. As a result, no small, clearly defined group of symptoms can be described which will entirely cover the effects of the disease on the host.

In general, however, the disease may be said to manifest itself by the loss of chlorophyll in more or less sharply defined areas of the leaf, by the production of a whitish down of conidiophores principally on the chlorotic area, and by a more or less extensive alteration in the form or the normal growth of the plant. The change in color is the most striking and obvious symptom. Since, however, somewhat similar changes in color and form may result from other causes, the characteristic downiness is the surest indication of the disease.

The effects of the disease may appear at any time from the putting out of the third or fourth leaf to the formation and maturing of the tassel and ear, but in any case the tissue of the host is thoroughly invaded by the mycelium before any external signs appear.

When appearing early in the development of the plant the symptoms are as follows: The second, third, or perhaps the fourth leaf, when nearly developed, shows at the base two or three rather narrow, longitudinal stripes of a pale yellow to whitish color (Pl. A) with the exception of which the leaf is quite the normal green. However, the two or three leaves already partly developed above this, and all the leaves which subsequently appear, are almost completely whitish or pale yellow. Moreover, these leaves never attain the normal shape and size but remain much narrower and become rigid, so that they ascend stiffly instead of bending in the normal flexible manner (Pl. A). The growth of the stem is also checked, so that the plant becomes more or less dwarfed. As the growth of the leaf sheaths is not decreased proportionately, they often deeply overlap to form a cover which may inclose and even project beyond the stunted tassel (Pl. 18, A). The root system also is usually affected so that

it does not develop properly but becomes stunted and functionally inadequate.

The subsequent fate of an infected plant varies with conditions. In the rainy season it almost invariably succumbs rapidly to secondary infections by species of *Pythium*, *Helminthosporium*, or *Fusarium*. In the dry season, however, although it usually turns brown, withers, and soon dies, such a plant may struggle along to the tasseling state and may even produce a stunted ear with occasionally a few grains.

When the disease appears later in the development of the corn plant the symptoms are as follows: The first leaf to show any signs of the disease, which may be the fourth or fifth or even up to the eighth, will have at the base pale stripes similar to, but more extensive and broader than, those described for plants which show the disease early (Pl. B). All subsequent leaves show a somewhat similar striping but in a progressively more marked degree, the markings on each successive leaf being more extensive than those on its predecessor and running nearer the tip, while the last leaves are striped throughout their entire length.

The shape of the stripes varies greatly. On the lower leaves they are usually merged at the base into a solid yellowish white area from which irregular elongations run up into the normal green towards the tip of the leaf (Pl. B). On the middle leaves the solid yellowish white area at the base is somewhat smaller in extent, but the prolongations from it run more nearly to the tip, while on the upper leaves these discolored stripes extend from the base to the tip of the leaf but are more broken and irregular and even merge laterally and anastomose so that a marbled or mottled appearance is given to the otherwise green leaf.

The shape and size of these leaves, however, is very little altered, and they usually have the breadth and flexibility which characterize leaves of the normal plant. At times, however, the midribs become brittle from the invasion of the fungus mycelium, break where they join the sheath, and hang straight down along the stem (Pl. 17). The growth and structure of the stem are often normal, and the root system is strong and well developed. It is in the reproductive structures of these later-infested plants that the injurious effect of the disease is shown especially. The tassel, although usually appearing at the normal time and often seemingly unharmed structurally, may show decreased production of pollen and frequently is extremely malformed (Pl. 17, A). The ear also is even more seriously affected. Even a mediocre ear is a very rare occurrence (in 1 out of 150 diseased plants), while customarily the ear is more or less completely sterile and malformed (Pl. 20).

This malformation of the reproductive structures is of frequent and regular occurrence in maize infected by the Philippine downy mildew. In plants attacked at all ages by the disease there is induced a great variety of the most remarkable malformations and monstrosities of the ear and tassel. These show a wide range of the fasciations, phyllodies,

reduplications, virescences, and other abnormalities of the various categories of monstrous growths that are recognized in teratology. Less frequently also the vegetative parts of the infected plants show abnormalities induced by the disease, fasciations and torsions of the stem (Pl. 17, B) and shank (Pl. 19, A) being most common. These abnormalities, of course, are frequently induced by other diseases and by unfavorable conditions of the environment, but their occurrence in connection with the downy mildew is so common as to form an accessory symptom of diagnostic value.

One other marked effect of the disease is the delaying of ear production. Normal plants in a plot invariably will bear well-developed ears in the "milk" or "glazing" stage before the diseased plants have developed ears to the "silking" stage.

It should be noted that the loss of chlorophyll and the consequent yellowish or whitish color of the marked areas, which is so characteristic a symptom of the disease, is by no means permanent but serves particularly to point out the earlier stages of the attack. As the diseased plant matures, however, and the fungus begins to terminate its period of spore production, the marked areas become more and more green, the contrast between the normal green and the paler portions of the leaf becoming less and less distinct until, finally, in plants less heavily attacked, the marked areas may so far regain their green color as to be almost indistinguishable from the normal.

All these plants which show the disease at a late date do not necessarily undergo rapid destruction as in the cases of early attack. On the contrary, although the plants are more susceptible to the secondary infections than are their healthy companions, they may mature along with them, drying and withering at a date only slightly earlier than normal. In some cases the infected plants seem stimulated by the downy mildew to prolonged activity and show persistent and excessive growth of husks, or of bracts in the deformed tassels, after adjacent plants are withered and dry (Pl. 19, A).

The susceptibility to infection is greatest in the young seedling and decreases markedly as the plant develops, so that by the time it has tasselled and is forming ears its tissue is, as a rule, too mature and resistant to permit infection. If, however, as is frequently the case in some varieties, the main plant sends out secondary shoots or suckers, these may rapidly become infected (Pl. 19, B), and through them the infection may spread to the main plant even though it is so far matured as to have its kernels hardening.

When attacked in this way, the mature plant shows symptoms different from any of those described above. The lower leaves are inconspicuously marked throughout their length with narrow, pale, yellow-green to rusty green stripes, which are not continuous but are irregularly broken and interrupted. On the middle leaves, as a rule, the markings

are similar in character but occupy principally the more distal part of the leaves, while the upper leaves are either entirely unmarked or have the striping confined to a small part of the leaf tip. Since most of the parts of the plant are matured, they show no change in form as a result of the infection, but the ear, if not mature, may elongate slightly and project from the husks at the tip (Pl. 19, B). It is to be noted that a plant thus attacked, in contrast to those previously described which are infected early, is marked least extensively and conspicuously on the upper leaves and most extensively on the lower leaves, has ears little if at all altered, and bears no conidiophores on the marked areas.

The production of conidiophores on the diseased plant is, of course, a symptom valuable in recognizing the disease (Pl. 21). Unfortunately, however, the process of conidiophore formation takes place almost exclusively at night and is controlled largely by conditions of the environment. The details of this relationship will be given later. It need only be said here that a plant may be attacked heavily by the fungus, the mycelium of which invades its tissues throughout, and may show the changes of color and growth which are characteristic of the disease and yet never form conidiophores and conidia unless external conditions are favorable.

A comparison of the symptoms of the Philippine downy mildew with those which characterize the downy mildew of maize in other countries shows many similarities.

In the closely related Javan mildew of maize, Palm (15) has recognized three distinct sets of symptoms. Of these, the symptoms of type A correspond in general to the description given above for plants attacked early in life, while the symptoms of type B correspond to those of plants attacked later. Type C, however, is characterized by narrow, inconspicuous stripes of a dark brown color running the full length of the lowest leaves and decreasing in extent on successively younger leaves until the last marked leaves show these stripes at the tip only, while the still later leaves are of the normal green throughout.

No specimens corresponding exactly to the description and illustrations of Palm's type C have been seen in the study of the Philippine maize mildew. Occasional stripings of this sort have been observed on the lower leaves of plants whose upper leaves showed the general or restricted discoloration already described. Maturing plants infected through suckers have shown inconspicuous, dark orange-colored stripings, extensive on the upper leaves and decreasing in area on the lower. In no case, however, has an immature plant been seen with these dark markings of the leaves decreasing on successively younger leaves until the latest are untouched.

For the Philippine maize mildew it does not seem justifiable to attempt to make such hard and fast categories as the types A, B, and C of Palm, although the symptoms shown by many plants can be more or less

roughly grouped under the types described above. The discolorations, growth changes, and other effects of the disease all differ markedly in accordance with time of infection, the varietal and individual character of the plant attacked, and the conditions of the environment. Hence there are encountered not only such diseased specimens as can be included conveniently in the three types recognized by Palm but also some plants which show symptoms intermediate between the types and others which show various combinations of these symptoms.

The occurrence of such sharply marked categories of symptoms as those described by Palm might with some justice be suspected to be the manifestation of different biologic strains of the causal fungus. In the Philippine maize-mildew, however, cross inoculations with spores from infected plants corresponding to Palm's symptom types, as well as biometric studies of the spores and conidiophores from these plants, disprove this assumption. Moreover, a series of experiments in which several varieties of maize were inoculated in various ways at different ages and subjected to different environmental conditions, although not entirely completed, has shown that the changes of color and growth produced in the plant by the disease differ with variations in these factors. All the evidence of field observations also supports this conclusion. In general, then, while the symptoms of the Philippine maize-mildew resemble those of the Javan, they appear to be much more varied and less easily grouped into sharply defined categories.

In the related Formosan downy mildew, Miyake (14) has described in detail only the symptoms shown by attacked sugar cane, which is the host most severely affected. He states that in maize the stripes are not particularly pronounced and the plant is not noticeably hindered in growth, for it ripens and shows only a slight decrease in yield. While this description would fit occasional plants attacked by the Philippine maize-mildew, it by no means depicts adequately the injurious effects in even the average case and would seem to indicate that the Formosan mildew is far less destructive to maize than is the Philippine.

Upon comparing the maize downy mildew of the Philippines with that of British India described by Butler (4), it is to be noted that the symptoms of the latter resemble in general those seen in the Philippines, although in the Indian disease more emphasis is laid on the checking of the internode growth and the consequent stunted appearance of the attacked plants than seems to be warranted from observation in the Philippines. Moreover, although the maize-mildew of British India has been present in that country since 1911, it has, in marked contrast to the Philippine disease, caused only slight sporadic injury.

HOSTS

Under field conditions throughout the Philippine Islands maize is the only crop on which the downy mildew occurs with sufficient severity to attract attention or to occasion appreciable loss. In the trial plots at

the College of Agriculture, however, where many kinds of cereal, forage, and cane crops were grown under conditions favoring infection, the downy mildew was found also to attack teosinte (*Euchlaena luxurians* Schrad.) and sorghum (*Andropogon sorghum* (Linn.) Brot.).

With teosinte, the percentage of infection and resulting loss is not quite so great as with maize, and the symptoms are less pronounced (Pl. 22, C), since the attacked individuals, especially those showing the disease late in their development, are much less conspicuously marked and are very seldom appreciably deformed, while the conidiophores are more scattered and more scantily produced.

As might be expected, hybrids resulting from the crossing of maize and teosinte are also susceptible to the disease, the degree of susceptibility and the effect on the plants attacked being intermediate between those shown by the two ancestors.

In sorghum the percentage of infection is very low, and the few plants infected are easily overlooked, because they turn pale when still very young (Pl. 22, B), bear but few conidiophores, and wither and die after a brief period of weak, stunted growth. No cases of individuals more conspicuously marked or deformed, in which the disease appeared later, were ever seen; and the loss was limited to the destruction of the few attacked plants.

Cross-inoculation experiments and the biometric study of spores and conidiophores show that the same causal fungus is involved in all these cases.

In view of this condition, it would naturally be suspected that other members of the Maydeae and Andropogoneae might also prove susceptible to the disease. So far, however, in spite of extensive search, no such *Sclerospora*, characterized by a conspicuous and rapidly spreading conidial stage, has been found in this region under natural conditions on the many wild grasses related to maize. However, the writer has found on *Saccharum spontaneum* L., a very common wild grass here, a *Sclerospora* which although of very frequent and widespread occurrence produces only the characteristic thick-walled resting spores. Further description of this *Sclerospora* will be given in a later paper, but it should be said at this point that this oogonial form on wild grass does not appear to be connected with the conidial form growing on cultivated maize, sorghum, and teosinte.

Moreover, inoculations such as were successful in the case of maize, sorghum, and teosinte have so far failed to accomplish the transfer of the disease to other related Gramineae—namely, *Coix lachryma-jobi* L., Philippine, United States, and Hawaiian strains; *Coix ma yuen*, Philippine and United States strains; several varieties of sugar cane (*Saccharum officinarum* L.), uba or Japanese cane (*Saccharum* sp.), and the native grasses, cogon (*Imperata cylindracea* L.), anias (*Andropogon sorghum* var. *halepense* L.), and aguingay (*Rottboellia exaltata* L.). In view of the

difficulties in securing artificial infection, these negative results are by no means conclusive, although the successful infection of maize, sorghum, and teosinte under the same conditions would seem to indicate that these other relatives are far less susceptible.

These inoculations will be detailed more fully in a later paper, but it should be said here that they were made from about 2 a. m. until dawn because the spore production was found to take place at this time. It seems highly probable that spore production is nocturnal in the other related downy mildews of the Orient as well and that the uncertain results of inoculations with them has been due to the failure to use fresh spores.

It is of interest to compare these results with those obtained for the other related downy mildews of the Orient. In Formosa, Miyake (14) successfully transferred *Sclerospora sacchari* T. Miy. from sugar cane to maize and teosinte and vice versa, but was unable to infect rice, sorghum, wheat, or millet. In India, although their infection experiments were unsuccessful, Butler (3) and Kulkarni (10) note the occurrence on teosinte of the conidial stage of a *Sclerospora* which they suspect may be identical with that of maize (*Sclerospora maydis* (Rac.) Butler.)

In Java, no extensive attempts were made by either Rutgers (19) or Palm (15) to obtain artificial inoculation of other hosts with the Javan downy mildew of maize (*Sclerospora javanica* Palm). They state, however, that under conditions favoring infection in the field, neither sugar cane nor the common wild alang-alang grass (*Imperata* sp.) was found infected and that, although teosinte itself is immune, the hybrid between teosinte and maize is, if anything, more susceptible than the variety of maize from which it is derived.

CAUSAL ORGANISM

The fungus which causes this extremely destructive disease of maize in the Philippines belongs to the Peronosporaceous genus *Sclerospora*, as Baker (1) and Reinking (17) already have reported. It should be noted, however, that it shows especially close relationship, not to the type species *Sclerospora graminicola* (Sacc.) Schroet., which is distinguished by the germination of the conidia by zoospores and the abundant production of oospores, but to those other oriental members of the genus which are characterized by the germination of the conidia by tubes and the partial or complete lack of oospores. However, setting aside the question of the affinities of the fungus for a later discussion, its characteristics will now be considered.

MYCELIUM

As a rule, as soon as the maize plant shows any external indication of the disease, the mycelium is found to be quite generally distributed throughout the host tissue, the root being the only main organ which is not extensively invaded. This invasion is most marked in the vegetative

parts of the plant but to a lesser degree affects the male and female inflorescences also.

Since the mycelium is relatively inconspicuous, its course throughout the host tissue is followed with difficulty. However, by means of transverse and longitudinal sections cut in various thicknesses and stained with iron-alum-haematoxylin and eosin or with gentian violet or methyl blue it was possible to trace the relation of the hyphae to the host tissue. Moreover, by subjecting such sections to the processes of maceration, clearing, and subsequent staining used by Mangin (13) and Berlese (2) the host tissue was readily dissociated and cleared sufficiently to permit the examination of large sections of the mycelium. By these methods material was studied from all parts of plants in various stages of infection, and the nature of the hyphae, their relation to the host tissue, and their location and abundance in different parts of the host were ascertained.

The hyphae are most abundant in the discolored areas of the infected leaves but may be found throughout the plant in unmarked parts of the leaves, in the branch tips of the apparently unaffected tassel, and at the base of the seemingly healthy stem some feet below the first discolored leaf.

In the leaf sheaths, leaves, and such modified foliar structures as the husks and glumes, the mycelium is most abundant among the cells of the bundle sheaths and in the mesophyll tissue (Pl. 23, E), but occasional hyphae are found in the fundamental tissue and even among the elements of the bundles themselves.

In the stem, ear shanks, cob, and tassel rachis, the mycelium follows the bundles, running for the most part parallel to them among the cells of the bundle sheath (Pl. 23, B) and less frequently sending out hyphae more extensively into the surrounding fundamental tissue.

In badly infected ears the mycelium usually runs out from the cob along the funiculus of attachment into the undeveloped parts of the abortive kernels, and occasional hyphae are encountered even in the chaff, seed coats, and endosperm of the apparently healthy kernels, though not in the embryo itself.

Wherever found, the hyphae are almost invariably intercellular in position, occupying even the smallest spaces between the cells, and even forcing adjacent cells apart as they grow between them. Occasionally hyphae were seen which apparently passed within the cells, but the interference of the host tissue was such that their position could not be ascertained with entire certainty. Since the size and shape of the hyphae are determined to a large extent by the nature of the intercellular spaces which they occupy, there is very little regularity in these characteristics in most cases. When separated by maceration, the hyphae are seen to be of two general types—namely, the long, slender, occasionally branching hyphae which lie alongside the vascular bundles in the stem and leaves'

and the lobed, contorted, irregularly branched, gnarled, and crooked hyphae which run in and out among the mesophyll cells of the leaves (Pl. 23, A).

The first kind seem to serve for communication from one part of the host to the other and can be followed for considerable distances even in longitudinal section (Pl. 23, B). The second kind appear to act as a means of establishing connection with the mesophyll cells, especially with the bundle sheath, in order to derive nutriment therefrom, since they are found in every possible crevice in the most intimate contact with the host cells (Pl. 23, A, E).

Haustoria are produced by both types of hyphae but are best developed or most pronounced on the crooked assimilatory hyphae among the mesophyll cells in the leaf. In shape the haustoria are simple, papillate to tubular (Pl. 23, F, G), as a rule, but they may be somewhat lobed (Pl. 23, H). In no case, however, were such markedly digitate haustoria seen as those figured by Rutgers (19, Pl. 6) for the Javan *Sclerospora*. The haustoria penetrate portions of the host cell wall, against which the hyphae are closely appressed, and project into the lumen. Not only the cells of the mesophyll, bundle sheath, and pith are penetrated, but also occasional cells of the epidermis (Pl. 23, E, c) and even the xylem (Pl. 23, E, b).

In any case, the haustoria accomplish the penetration of the host cell without occasioning its collapse, although the wall often is wrinkled and the turgidity of the cell decreased, apparently through the extraction of its contents by the parasite. The chloroplasts of the parasitized cells are gradually destroyed through the action of the fungus, with the result that the badly infected areas lose their green hue and assume the pale yellow or whitish color symptomatic of the disease. Occasionally the host cell surrounds the haustoria of the parasite with a thick wall (Pl. 23, F), as if in protective response to the injurious stimulus of the fungus, a condition observed also by Butler (3) in *Sclerospora graminicola* (Sacc.) Schroet. on *Pennisetum*.

The hyphae are hyaline, rarely if ever septate, thin-walled, with granular content, and vary greatly in size, $8\ \mu$ being perhaps the most common diameter. The haustoria are similar in structure and usually about $8\ \mu$ long by $2\ \mu$ in diameter.

In the larger air chambers which underlie the stomata, the mycelium develops somewhat irregular clusters of stout branches (Pl. 23, E, a), from which, under favorable conditions, the conidiophore initials arise and grow out through the stomata to produce the conidiophores.

CONIDIOPHORES

Conidiophores may be said, in general, to be produced on any part of the plant save the roots. They occur on the main stem, on the leaves, leaf sheaths, and ear husks, and on the main axis, branches, and glumes

of the tassel. Most commonly, however, the conidia appear on the leaves and leaf sheaths, where they occupy principally the conspicuous mottled and discolored areas which have been described.

On whatever part of the plant they may be found, the conidiophores emerge at night, provided there is present a thin layer of dew, rain, or mist. Damp air alone does not seem to permit their formation. Under favorable conditions the process of conidiophore emergence and conidia production begins about midnight and may continue a few hours after dawn, provided the weather is favorably rainy. When seen at night in the luxuriance of their growth, the innumerable conidiophores projecting slightly from the thin film of moisture on the leaves form a very distinct grayish white down, which is by no means even suggested by the dry, matted fragments which remain when the hot morning sun has dried the surface of the leaves (Pl. 21, A).

This process of conidiophore development and conidia production has never been described, and, since it shows several points of interest, it will be presented in detail in a subsequent paper. In general, however, it occurs as follows:

From the stomata of the infected portion one or more club-shaped hyphae grow out. These elongate, and under favorable conditions the paired protrusions finally bud out from their tips and become the stout primary branches. From the tips of these in turn bud out the beginnings of the secondary, and from these, at length, the tertiary branches, each of which usually terminates in one or two tapering sterigmata. Since the initial protrusions which develop into the branches arise almost invariably in pairs, the structure of the mature conidiophore is characteristically dichotomous, instances of the suppression or delayed formation of a branch being, on the whole, rather rare (Pl. 24, D). Finally, from the tip of each sterigma there buds one conidium as a spherical protrusion which enlarges and lengthens until it attains the elongate oval or rounded oblong shape of the mature spore and is separated from the sterigma tip by a cross wall.

When fully formed the conidiophore appears as in Plate 24, C, and consists essentially of a main axis which begins with an elongate basal cell and broadens gradually until it divides into the two to four stout main branches. From each of these extend two to four smaller secondary branches, each of which in turn bears two to four tertiary branches that terminate severally in one or two tapering sterigmata, each bearing at its tip a conidium. Although under favorable conditions the conidiophores are of the large, well-developed type just described, they frequently show such variations in structure as the omission of the second and third series of branches and a general reduction in branches, sterigmata, and consequently number of conidia (Pl. 24, E). On vigorous conidiophores 32 to 96 conidia may be borne, while on poorly developed ones there may be as few as 8 or even 3. In size also there is great varia-

tion, the total length of the conidiophore even in abundant dew varying from 260 to 400 μ , although most commonly it is about 340 μ , while in scanty dew, such as occurs in the hot season, lengths of 160 to 200 μ are generally encountered. In either case, however, the greatest width, just below the branches, is from 15 to 26 μ . The sterigmata are consistently about 10 μ in length, with a diameter at the base of about 6 μ .

The basal cell is invariably present in the mature conidiophore, forming a structural feature which should be emphasized as distinctive (Pl. 24, H, J, L). This cell reaches its greatest width at the septum which separates it from the rest of the main axis and tapers gradually downward throughout its length, terminating in a rounded, slightly swollen foot which is connected by a slender hypha with the internal mycelium through the stomatal pore. The greatest width of the basal cell is usually about 12 μ , but the length varies from the customary extremes in heavy dew (60 to 120 μ) to 30 or even 20 μ in a scanty film of moisture (Pl. 24, E).

When fully mature the conidia are most commonly elongate, ellipsoid, elongate ovoid, or rounded cylindric in shape, are thin-walled and hyaline, and have a more or less finely granular content. The tip is broadly rounded and lacks any papilla or other modification, while the base shows an apiculus, a slight thickening and protrusion of the wall at the point of attachment to the sterigma. Wide variations in the shape of the conidia are common, examples being found of all of the types from subspherical, pyriform, or even lemon-shaped to the extremely elongate types which are shown in Plate 25, C-L.

A method has been devised by Rosenbaum (18) for expressing quantitatively the shapes encountered in a study of large numbers of conidia of *Phytophthora*. This method, which consists in classifying and plotting the ratios of length to width, is of value in that it gives a quantitative idea of the relative predominance of certain shapes of conidia in a species and furnishes a reliable basis for comparison with others. Unfortunately, however, this method can make no distinction between conidia which are ovate or obovate, pyriform, or obpyriform, ellipsoid, allantoid, or cylindrical, provided their length and greatest diameter be the same. Therefore, while the ratios of length to width of 400 conidia of the Philippine *Sclerospora* of maize are presented here in tabular and diagrammatic form for comparison with other species, a clearer idea of the variations in shape is probably to be obtained from the figures in Plate 25.

The size of the conidia also varies greatly. When large numbers are examined, examples are found with such widely different dimensions as to include those given for several other species. It is difficult, therefore, to give a correct impression of the size of the conidia by means of the extreme dimensions within which they vary, or even by means of the average dimensions. However, the method of grouping together large numbers of representative conidia into a series of measurement classes and plotting curves to show their frequency of occurrence has been used

successfully in describing the size of similarly variable bodies, first by Rosenbaum (18) for *Phytophthora* and more recently by Gaumann (6) for *Peronospora*. This method seems especially valuable in the case of such variable structures as the conidia of the *Peronosporaceae*, since by means of it data gathered from large numbers of individuals may be so presented that the range of variation in size which is encountered, as well as the size class which predominates in the species, is at once apparent. Also it furnishes a most accurate method for comparing the sizes of such bodies in different species.

TABLE I.—Measurements and ratios of length to width of 400 conidia of *Sclerospora philippinensis* arranged in classes.

Number of conidia in 400.	Length classes.	Number of conidia in 400.	Width classes.	Number of conidia in 400.	Ratio of length to width classes.
	μ .		μ .		
1	17 to 18.9	1	11 to 12.9	1	1.05 to 1.14
1	19 to 20.9			0	1.15 to 1.24
2	21 to 22.9	8	13 to 14.9	3	1.25 to 1.34
1	23 to 24.9			6	1.35 to 1.44
4	25 to 26.9	41	15 to 16.9	9	1.45 to 1.54
10	27 to 28.9			30	1.55 to 1.64
35	29 to 30.9	160	17 to 18.9	53	1.65 to 1.74
68	31 to 32.9			59	1.75 to 1.84
75	33 to 34.9	148	19 to 20.9	70	1.85 to 1.94
64	35 to 36.9			59	1.95 to 2.04
55	37 to 38.9	41	21 to 22.9	40	2.05 to 2.14
30	39 to 40.9			32	2.15 to 2.24
24	41 to 42.9	1	23 to 24.9	21	2.25 to 2.34
21	43 to 44.9			9	2.35 to 2.44
7	45 to 46.9			2	2.45 to 2.54
2	47 to 48.9			3	2.55 to 2.64
0	49 to 50.9			0	2.65 to 2.74
1	51 to 52.9			3	2.75 to 2.84

For these reasons this method seems well adapted to depict the size of the conidia of the *Sclerospora* of Philippine maize. Accordingly the measurements of 400 conidia are given in tabular form and are also plotted as curves (fig. 1, 2). The ratio of length to diameter in classes is given in figure 3. These show clearly that while spores are encountered with such widely differing dimensions as 18 μ long by 12 μ in diameter, and 51 by 23 μ , the size which predominates is 34 μ by 18 μ , and by far the greater number of spores encountered are from 27 to 39 μ in length by 17 to 21 μ in diameter. Although these measurements are of conidia produced on maize, they have been compared and found to agree with similar measurements of conidia from teosinte and sorghum. On comparing like tabulations of dimensions of fresh conidia with those from material mounted in glycerin or dried, the writer finds constant slight differences, particularly in width. Therefore, these 400 measurements were made on four occasions at the beginning of the period of maximum conidia production (2 to 3 a. m.) from fresh material mounted in dew

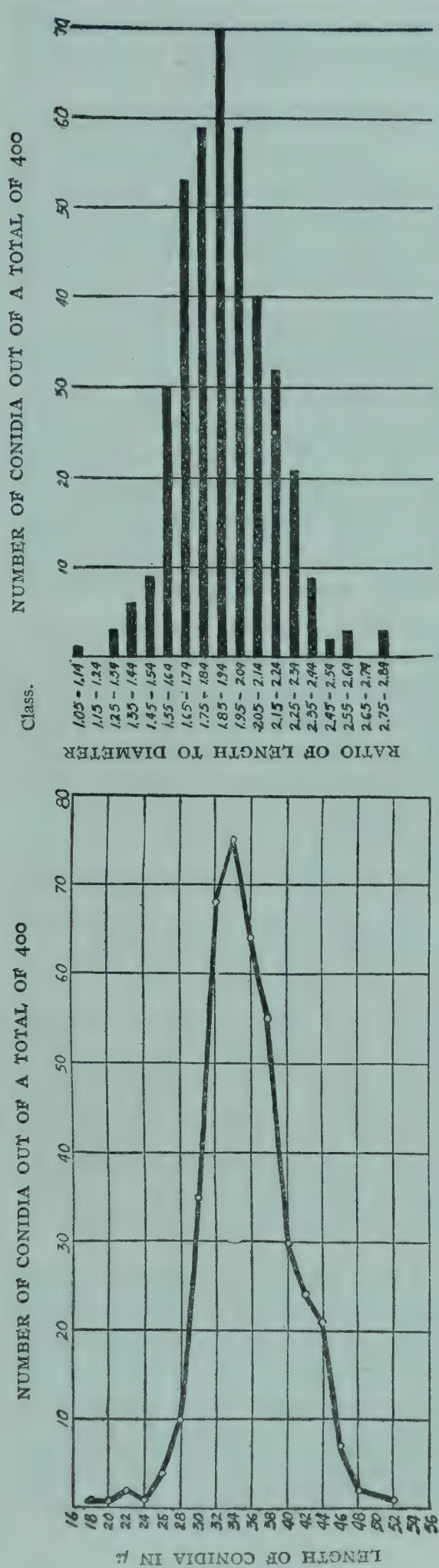


FIG. 1.—Graph showing variation in length of conidia of *Sclerospora philippinensis*.

FIG. 3.—Diagram showing ratios of length to width of conidia of *Sclerospora philippinensis*, arranged in classes, and indicating limits of variation and mode.

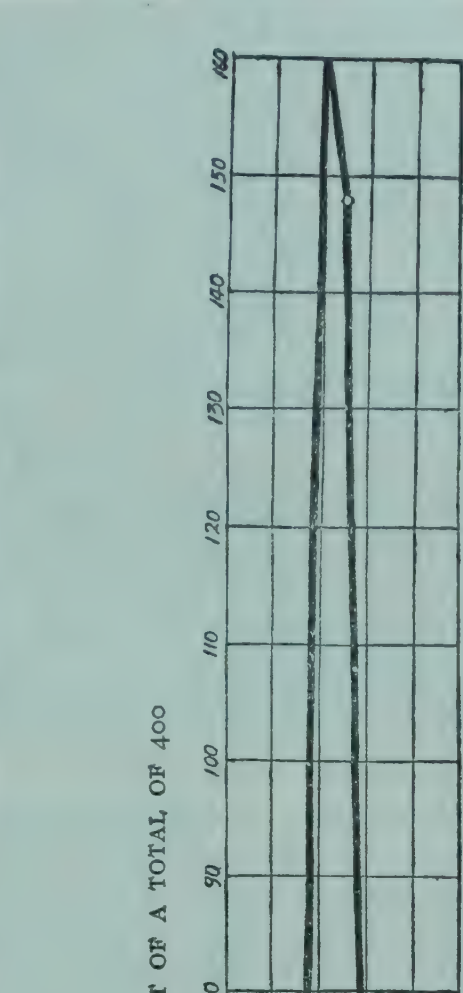
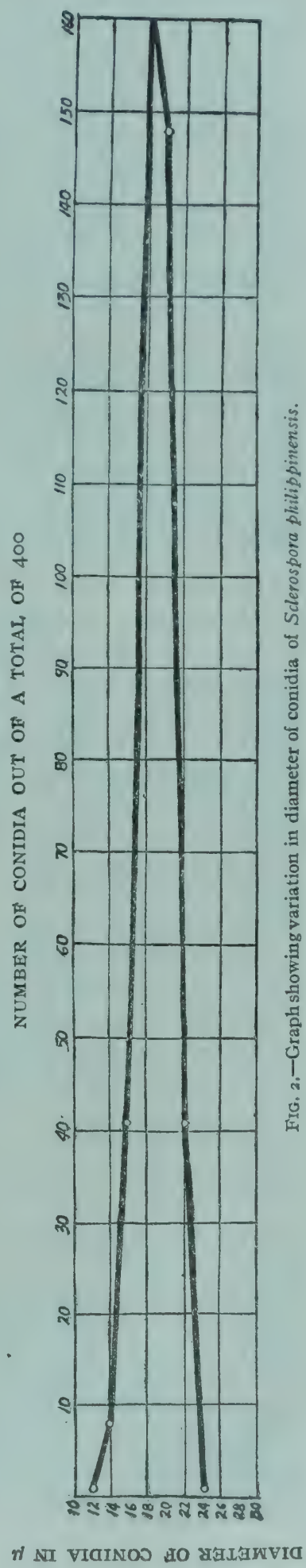


FIG. 2.—Graph showing variation in diameter of conidia of *Sclerospora philippinensis*.



or rain water, and they probably furnish an expression of the conidia dimensions and a means of comparison with other species which is as accurate as it is possible to obtain.

It is interesting to note that occasional monstrous conidia were seen, resembling somewhat those described by Miyake (14) for *Sclerospora sacchari*. Since these have the same structure and history as those of more usual size and appear to represent merely the upper extreme of the widely varying conidia dimensions, they are regarded by the writer as of no special significance.

Germination of the fresh conidia takes place readily in dew, in rain water, in water from clear brooks, in dilute nutrient solutions of various kinds, and on similar solutions solidified with 1 per cent agar. When once the conidia are dried, however, they will no longer germinate under any conditions. On the moist surfaces of newly infected plants in the field, large numbers of conidia may be found germinating vigorously at any time from about 3 a. m. until dawn, but after the rapidly drying effect of the early sun has been felt for one or two hours there can be found on the same plants only shrivelled spores incapable of further development. Germination is preceded by a swelling and consequent alteration in size and shape of the conidium and invariably proceeds by the protrusion of one or more germ tubes (Pl. 25, C-L). This may take place from any part of the spore, and the hyphae thus produced may simply elongate (Pl. 25, C, J) or develop variously into extensively branching systems (Pl. 25, E, F, L). Occasionally the hyphae of germination grow up into the air for a short distance and produce at their tips an ovoid swelling that might perhaps be interpreted as an abortive attempt at a secondary conidium formation such as has been found in other Peronosporaceae. In no case was the production of zoospores by the conidia observed, although repeated attempts were made to induce this method of germination.

In spite of the ease with which the conidia produce germ tubes, all attempts to induce continued independent development of the mycelium in artificial media have been unsuccessful, the growth seemingly ceasing when the nutriment of the spore is exhausted. In view of the tropical habitat of this *Sclerospora* it is of interest to note that the conidia germinate readily when maintained at a temperature as low as 6.5° C., even though the temperature at which they most commonly germinate is from 20 to 24°.

In spite of extensive search, none of the resting or resistant bodies customarily encountered in this or other genera of the Peronosporaceae, such as chlamydospores and parthenogenetic or normal oogonia, have ever been found to be associated with the conidial stage of this fungus. Every effort has been made to find such structures. The progress of the disease has been observed in individual plants from the time of their infection by the fungus to their ultimate disintegration in many varie-

ties representing the several types of maize and teosinte and in sorghums under all the various conditions of the wet, dry, and transitional periods of the year. Furthermore, infected plants have been subjected to various changes in temperature, moisture, light, aeration, and soil, in the attempt to induce the formation of such structures. So far, all efforts have been in vain, and, although facilities were not available for any such experiment as subjecting the infected plants to long-continued cold or total freezing such as might occur in our own corn belt, still the experiments which were made seem to indicate that the formation of resting bodies by the fungus in maize occurs very rarely if at all under the conditions naturally encountered in the Philippine Islands.

The possibility that the conidial stage may be restricted to maize while the production of oogonia takes place on some other host invites consideration. As has been mentioned above, the writer has found a *Sclerospora* attacking a common field grass, *Saccharum spontaneum*, in this region; but whether this fungus, of which only the oogonial stage has been seen, is in any way connected with the conidia-bearing *Sclerospora* on maize remains to be determined.

IDENTITY OF THE CAUSAL FUNGUS

The important question of the identity of this Philippine *Sclerospora* necessitates a comparison with the other members of the genus. Since our knowledge of the Philippine form is at present confined to its conidial phase solely, no comparison is possible between it and those species of which only the oogonial stage has been recorded, such as the remarkable *Sclerospora magnusiana* Sor. (20) of *Equisetum* from Russia, the rare *Sclerospora farlowii* Griff. (7) of *Chloris* from western North America, the recently described *Sclerospora miscanthi* T. Miy. (14) of *Miscanthus* from Japan, or even the more common *Sclerospora macrospora* Sacc. (8), which is widely distributed on a large number of grasses and even has been found on the tassels of maize in Italy. Likewise, the type species *Sclerospora graminicola* (Sacc.) Schroet., although known from all over the world on a wide range of wild and cultivated grasses and even recorded on maize in Argentina (21), can not be directly compared, because the conidial stage, even though known, is rare and is characterized by the germination of the conidia by zoospores and by the invariable predominance of the typical oospores.

A far closer relationship is shown between the Philippine form and those Oriental species which occur on maize or related gramineous hosts and are characterized by the partial or complete lack of an oogonial stage, with the concomitant predominance of the conidial phase, which is distinguished further by the germination of the conidia by tubes.

Of these there have been enumerated the following: *Sclerospora javanica* (Rac.) Palm, of Java (originally described by Raciborski as *Peronospora maydis*); *Sclerospora maydis* (Rac.) But., of India; and

Sclerospora sacchari T. Miy., described by Miyake from Formosa but reported by Lyon (11, 12) also in the Fiji Islands and Queensland.

It has been assumed by Baker (1) and Reinking (17) that the Philippine *Sclerospora* of maize is identical with *Sclerospora maydis* (Rac.) But. of India, and this has been generally accepted by other investigators. Since no detailed description of the species with critical measurements has been published, and the single conidiophore and few spores figured by Reinking are hardly enough on which to base a decision, it seems necessary to corroborate the identification of the fungus.

A comparison with *Sclerospora maydis* (Rac.) But. of India and also with the other related species mentioned above is accordingly in order. Such a comparison must necessarily consider the field characters of the disease, such as its effect on the plants attacked, its severity, and its fatality to the various hosts, as well as the specific peculiarities of the causal organism itself. Of these the characteristic structure and dimensions of the organism itself are most valuable, since the field characters show, on the one hand, a general similarity in all these fungi and, on the other hand, vary so widely under different conditions as to be confusing even in one species.

The *Sclerospora* causing the Philippine disease is known in its conidial phases only, and a comparison of this form with other species must be based on this stage. Such a comparison is confronted by many difficulties. In the first place, the characters most valuable from the systematic point of view have been found by the writer to vary greatly under different conditions and at different stages in the development of the Philippine form, and they probably do so in the other forms also. For instance, the very important characters of the size and shape of the conidia and the structure and dimensions of the conidiophores vary greatly at different stages of development and under different conditions.

The conidia begin as small spherical outgrowths from the sterigmata tips, and in their development become larger and more elongate, passing through ellipsoid (Pl. 24, A), oval (Pl. 25, A), and even pyriform stages before they eventually assume the elongate ovoid ellipsoid or rounded cylindrical shape of complete maturity (Pl. 24, C). They are then separated from the sterigma tip by the septum.

This characteristic shape is transient, however, for, after they are free from the conidiophores, the spores show an almost immediate imbibition of moisture, which results in a marked increase in size and in a more rotund shape, due to the greater bulging of the side walls. Moreover, the apiculus which marks the point at which the spore was attached to the sterigma is modified, by the swelling of the spore, to a low, rounded curve.

Since the partially developed spores of various shapes and sizes may be detached from the sterigmata and still retain their contents and germinability, and since marked changes from the shape and size of the

mature spores normally follow when it is free, it is obvious that a mount of spores usually comprises a motley collection of shapes and sizes, only a comparatively small number of which represent the characteristics of the normal and mature spore.

Moreover, aside from these variations which mark the normal development of the spore, there are also changes in size and shape resulting from abnormal conditions such as the sudden checking of development by unusual drying of the necessary layer of moisture on the leaf.

The size and structural characteristics of the conidiophores also vary markedly with attendant environmental conditions. The normal order is for primary, secondary, and tertiary branches to form before the sterigmata develop and begin to bud out the spores. If the gradual drying of the film of moisture on the leaf surface begins to check this process before its completion, however, sterigmata formation and spore production ensue prematurely, and conidia may be borne on the secondary or primary branches of the conidiophore (Pl. 24, I), or even on the apex of the main axis itself. Similarly, the growth of the basal cell and main axis may be curtailed (Pl. 24, E). Obviously, as a result of these changes, the height of the conidiophore shows a corresponding alteration.

Finally, after it has lost its conidia, the conidiophore shrivels and is dried to an almost unrecognizable mummy by the morning sun.

Since it appears highly probable that similar variations in size and structure occur also in the other oriental mildews, it is difficult to make any adequate comparison from the data available. To permit accurate comparison one should have descriptions and illustrations of material, or the material itself, collected under the optimum conditions, which in the case of the Philippine downy mildew occur on cool nights with heavy dew or persistent rain from 2 to 4 a. m.

In the light of this fact, Miyake's (14) data are valuable, as he recognized that conidiophores and conidia were produced at night, and his drawings show that he illustrated excellent material. Most investigators, however, failed to realize this, and their material, as their descriptions and drawings show, was inadequate and scanty.

When one compares the available data, inadequate though they be, the following points are apparent. The Philippine and Javan *Sclerosporas* are alike in that the conidial phase is the only one yet known.

The conidiophores of the former closely resemble those of *Sclerospora javanica* Palm both in size and structural characteristics, such as the basal cell, the main axis, the branch system, and the ultimate sterigmata. On the contrary, the conidia of the two forms are noticeably different. In the Javan fungus they are oblong rotund in shape and measure 19 to 26 μ in length by 15 to 20 μ in diameter, while in the Philippine mildew they are elongate ellipsoid, elongate oval, or rounded cylindric and markedly longer, most of the conidia encountered measuring about 34 μ in length

by $17\ \mu$ in diameter, and comparatively few showing the shortness which marks the Javan form. Moreover, although the field characters of the two diseases are very similar, the Javan *Sclerospora* presents an additional point of difference in that it does not attack teosinte, although teosinte-maize hybrids are, if anything, even more susceptible to it than maize itself (19).

To *Sclerospora sacchari* T. Miy., of Formosa, the Philippine maize mildew shows a very close resemblance in the size, the form, and even the minor structural characteristics of the conidiophores. Also, the conidia of the two forms are evidently quite similar, since the illustrations and the description (ellipsoid or oblong with rounded apex, 25 to $41\ \mu$ long by 15 to $23\ \mu$ in diameter) of the Formosan conidia are applicable to those of the Philippine species also. A marked difference between the two, however, is shown in their virulence on various hosts, for, while *Sclerospora sacchari* grows on both maize and teosinte as does the Philippine *Sclerospora*, still the former attacks sugar cane of many varieties, including those grown most commonly in the Philippine Islands, with violent intensity, while the latter, so far as is known, does not infect that crop at all. In the Philippines, in regions heavily infected with the maize-mildew, sugar-cane fields comprising many varieties grown under widely varying conditions and situated adjacent to the badly infected maize, and even containing some maize plants growing among and in contact with the young cane, have been under frequent observation during all stages of their development for over a year, and yet no case of infection with the downy mildew of maize has ever been seen.

Moreover, inoculation experiments such as were successful with maize, teosinte, and sorghum have so far failed to cause infection of the Philippine *Sclerospora* of maize on sugar-cane varieties found susceptible to the Formosan disease. Furthermore, the oogonial stage which has been reported for *Sclerospora sacchari* T. Miy. forms an additional point wherein it differs from the Philippine fungus, although it should be noted that the oogonia, which have been found only once and are not figured, have not been proved to be connected with the conidial stage of *Sclerospora sacchari*.

On comparing the Philippine downy mildew of maize with the British Indian species (*Sclerospora maydis* (Rac.) But.), with which it has been regarded as identical, a close resemblance indeed is apparent. The conidia especially are similar in both shape and size in so far as one can judge from the data available; the lack of any other type of spore is another point of agreement. In considering the conidiophores of the former, however, it should be noted that the description, dimensions, and illustrations indicate that the material was imperfect, for if one may judge from the Philippine fungus, the size and the abruptly ending base of the conidiophore signify that the main axis had been broken off just above the basal cell. Any accurate comparison, therefore, is difficult. The

sterigmata, however, are comparable, and it is clear that those of the British Indian fungus are markedly larger (15 to 20 μ long) than those of the Philippine species.

Moreover, the field characteristics are noticeably different. Although Butler reported the first attack of the disease at Pusa in 1912 and emphasized the probability of its spreading to other fields of the region, his latest report (5) indicates that it has continued to be only slightly and restrictedly destructive, an effect markedly in contrast to the rapid spread and serious damage of the Philippine fungus. Also, Butler's description emphasizes the stunting of the growth and resultant bunched appearance of the plant as a characteristic feature of the disease in India, while in the Philippines this is but one and certainly not the most striking effect of the disease.

While the matter is necessarily unsettled because of lack of adequate description of the British Indian form, certain points would seem to indicate that the maize-mildew of India is a different physiological variety and probably a different species from that of the Philippines. These points are the differences in the causal fungi and the symptoms, and especially the lack of virulence shown by the Indian disease and its failure to spread through Bengal where "maize is a crop of considerable importance" and where the conditions of climate and culture are little if at all different from those of some infested regions of the Philippines.

In any case, however, it should be noted that the name *Sclerospora maydis* (Rac.) But. is not strictly a tenable one, for it was applied to the British Indian maize-mildew by Butler (3, p. 15) on the assumption that it was identical with the Javan. Butler (4, p. 275-276) concluded from his comparison with the diagrammatic drawings and incomplete descriptions of Raciborski (16) that the downy mildew of maize in British India—

was found to be identical with the one which causes great damage to this crop in Java,

and that—

its cause is a fungus named *Peronospora maydis* by Raciborski.

The more recent and extensive work of Palm (15), however, has shown clearly that the Javan fungus, although indeed a *Sclerospora*, is a distinct species, one which Palm names *Sclerospora javanica*. This leaves *Sclerospora maydis* (Rac.) But. as the name of the British Indian maize-mildew.

Therefore, because the points of difference already considered seem to indicate that the downy mildew of maize in the Philippines is not identical with the one in British India, and because the name *Sclerospora maydis* (Rac.) But., given to the latter, is technically untenable, it seems necessary to distinguish the Philippine downy mildew of maize. Hence it is

given the name of *Sclerospora philippinensis*, n. sp., with the diagnosis as follows:

***Sclerospora philippinensis*, n. sp.¹**

Sclerospora Maydis, Reinking, 1918, in Philippine Jour. Sci., s. A, v. 13, no. 5, fig. 39, pl. 20, fig. 1-2, not Butler.

Forming linear or irregular whitish yellow to pale spots, often entirely discoloring the leaves and more or less deforming the host.

Mycelial hyphae growing intercellularly in all parts except the root, branched, slender, usually about 8 μ in diameter, but irregularly constricted and inflated, haustoria simple, vesiculiform to subdigitate, small, about 8 μ long and 2 μ in diameter.

Conidiophores always produced in night dew and growing out of the stomata, erect, 150 to 400 μ long, 15 to 26 μ thick, bearing a basal cell in the lower part, dichotomously branched two to four times above, branches robust, sterigmata conoid to subulate, 10 μ long, slightly curved.

Conidia elongate ellipsoid, elongate ovoid, or rounded cylindrical, varying in size, usually 27 to 39 μ long by 17 to 21 μ broad, hyaline, with thin episporium, minutely granular within, slightly rounded at the apex, provided with a minute apiculus at the base, always germinating by a tube.

Oospores not yet seen.

Material of the type has been deposited in the pathologic collections of the Bureau of Plant Industry, Washington, D. C., in the Cryptogamic Herbarium at Harvard University, Cambridge, Mass., and in the herbarium of the Bureau of Science, Manila, P. I.

So far as at present known there exist in the Orient the following *Sclerosporas* which are of primary importance, since they cause serious diseases of maize.

Sclerospora javanica Palm, known on maize and maize-teosinte hybrids in Java, Madoerah, and Sumatra.

Sclerospora maydis (Rac.) But., known on maize and teosinte in Bengal, British India.

Sclerospora sacchari T. Miy., known on maize, sugar cane, and teosinte in Formosa, and on sugar cane in Queensland and the Fiji Islands.

Sclerospora philippinensis, n. sp., known on maize, teosinte, and sorghum in the Philippine Islands.

All these species are very similar in their effects and show close relationship in structure and development. All are characterized by the

¹ *Sclerospora philippinensis*, sp. nov.

Maculas lineares vel irregulares, albido-flavas vel pallidas efficiens, saepe totum folium discolorans, et matricem plus minusve deformans.

Hyphis mycelicis intercellulas in totas partes praeter radicem crescentibus, ramosis, tenuibus, plerumque circa 8 μ in diametrum, sed irregulariter constrictis inflatisque, cum haustoriis simplicibus, vesiculiformibus subdigitatisve, minutis, circa 8 μ longis et 2 μ in diametrum ornatis.

Conidiophoriis semper in rore nocturno productis, e stomatibus egredientibus, erectis, 150-400 μ longis, 15-26 μ crassis, in parte inferiore cellulas basiales gerentibus, superne 2-4 dichotomo-ramosis, ramis robustis cum sterigmatibus conoideo-subulatis, 10 μ longis, leviter curvatis.

Conidiis elongato-ellipsoideis, elongato-ovoideis vel rotundato-cylindraceis, variis dimensione, plerumque 27-39 μ longis et 17-21 μ latis, hyalinis, episporio tenue, intus minute granulosis, apice leviter rotundatis; basi cum apiculo minute munitis, semper per tubum germinantibus.

Oosporis nondum visis.

Hab. in foliis, vaginis, glumis, bracteis, culmis, et inflorescentiis praecipue *Zae maydis*, rarius *Euchlaenae luxuriantis* et *Andropogonis sorghi* per omnes partes in insulis Philippinis.

predominance of the conidial stage, no oospores having been found connected with any save *Sclerospora sacchari*, with which, indeed, the relationship is not very well established.

Furthermore in all these species the conidiophores are large and prominent with a differentiated basal cell, stout main axis, and extensive dichotomous system of branches comprising large primary, secondary, tertiary, and even quaternary branches. The germination of the conidia also is invariably by means of hyphae.

In contrast to these species the cosmopolitan *Sclerospora graminicola* (Sacc.) Schroet., the type on which the genus was established, is characterized by the predominance of the oogonial stage, the conidial phase being comparatively rare; by its smaller inconspicuous conidiophores, which lack a differentiated basal cell and give rise to few short primary or at times secondary branches only; and by the regular germination of the "conidia" by zoospores.

Such marked and essential differences certainly appear to indicate that these oriental species should be separated from the type as a different genus; but, in the opinion of the writer, such a step can not be made with justice until more is known of the conidial stage of *Sclerospora graminicola* and of the oogonial stage of the oriental forms.

Moreover, whether *Sclerospora graminicola* var. *andropogonis-sorghii* Kulk. should be included with the oriental group by virtue of its well-developed conidiophores and the germination of the conidia by hyphae, as Ito (9) suggests, also depends on further knowledge of the points just mentioned.

When one considers the great variations in effect on the host and even in such essential features as the characteristics of the conidiophores and conidia, which have been found by the writer to occur in *Sclerospora philippinensis* under different conditions of the environment at different stages of its development and on various hosts, one can not avoid a suspicion that these oriental forms may in reality be a single species. It is not inconceivable that such may be the case and that the variation in effect on the host, the susceptibility of different plants in different places, and the variations in structure of the causal organism may all be due to environmental conditions of the regions in which they are found. Obviously to settle these important points conclusively there is need of extensive cross-inoculation experiments and of comparative studies, using optimum material and methods which emphasize important characters quantitatively as well as qualitatively.

The problems of the origin of these destructive *Sclerosporas* of maize and of their geographic distribution, their appearance in the Orient where maize has only been introduced since about 1496, and their absence as yet from the Western Hemisphere where maize originated, are all too involved for consideration at present.

In any case, however, the increasing attention which these dangerous downy mildews of maize have demanded by their destructive activity throughout the Orient in recent years must necessarily arouse the apprehension of all who are concerned with the valuable corn and sugar-cane interests of the United States.

SUMMARY

(1) For several years there has been known to occur in the Philippine Islands a destructive downy mildew of maize, which not only causes serious losses in that region but also threatens our own valuable corn crop, should it reach the United States. Prior to investigations by the writer no extensive study of this disease has been made. This paper presents certain results in regard to the distribution, severity, and characteristics of the disease and the nature and relationships of the causal fungus.

(2) The disease occurs throughout the Philippine Islands, where it evidently has been established for some years.

(3) It is extremely destructive. Under favorable conditions whole fields are destroyed, and in some districts it has even forced the natives to abandon corn culture entirely.

(4) Representative varieties of all types of maize are highly susceptible, and teosinte, maize-teosinte hybrids, and sorghum are attacked, but with less virulence. Inoculation experiments on a number of related plants, both wild and cultivated, gave negative results.

(5) Symptoms of the disease may appear from the time the plants are seedlings with three or four leaves to the time the tassels and silk are developed. In general, infected plants show a yellowing of the leaves in more or less restricted striped areas, a whitish down of conidiophores, principally on the leaves, abnormalities in growth of the vegetative parts, and abortive development of the ear, resulting in partial or complete sterility. These effects of the disease are described and illustrated.

(6) The causal fungus belongs to the genus *Sclerospora* of the Peronosporaceae and is characterized by the predominance of its conidial stage, the lack of oospores, so far as known, and the invariable germination of its conidia by hyphae. In these respects it differs from the type species *Sclerospora graminicola* (Sacc.) Schroet., which is distinguished by its evanescent conidial stage, its predominating oospores, and the germination of its "conidia" by zoospores. The Philippine species shows close relationship to the following recently described oriental species, all of which attack maize: *Sclerospora javanica* Palm, of Java, *Sclerospora maydis* (Rac.) But., of British India, and *Sclerospora sacchari* T. Miy., of Formosa, Queensland, and the Fiji Islands.

The Philippine *Sclerospora* appears to be a new species and is described as *Sclerospora philippinensis*, n. sp.

(7) Maize plants usually are infected as very young seedlings, and less often as they mature. In any case, however, when the symptoms appear, the mycelium of the fungus already has invaded the host tissue extensively. The mycelium may be found in practically every part of the maize plant with the exception of the root, but is most abundant among the bundle sheath cells of the leaf.

(8) The conidiophores are produced in vast numbers but only at night when a thin layer of dew or rain is on the leaf surface. They vary greatly in size and development according to the depth and persistence of this layer. These variations are described and figured.

(9) Since the conidia also show wide variation in size and shape, an attempt is made to give a quantitative idea of this by tables and graphs of the measurements of 400 specimens. When fresh, the conidia germinate readily in water and various culture media at temperatures ranging from 6.5° to 25° C., and invariably by hyphae. Once they have become dried the conidia no longer germinate; hence their distribution and the infection of new plants occurs almost always before dawn.

(10) In spite of extensive search, no oospores or other resting bodies have yet been found to be produced by this *Sclerospora*. It apparently maintains itself by transmission from plant to plant. The writer has found the oospore stage of a new *Sclerospora* on *Saccharum spontaneum* L., a common wild grass of the Philippines. Whether this is in any way connected with the conidial stage on maize remains to be determined.

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PLATE A¹

Young maize plant, showing the effects of a very early attack of the downy mildew on a small, early maturing variety, Manobo Yellow. Notice the dwarfing and the pale appearance of the plant as a whole, the narrowness and stiffness of the leaves, and the narrow striping of the later leaves throughout their length. The plant was 32 days old when photographed and had first shown the disease two weeks after emerging from the soil. One-fourth natural size.

¹ In the preparation of Plates A and B the diseased plants were photographed and enlargements were colored to correspond as closely as possible to the living specimens. Prepared by L. S. Weston.

PLATE B

Young maize plants, showing the effects of later attack of the downy mildew on a large, late-maturing variety, Guam White Dent.

The two plants at the right are diseased; the one at the left is healthy. Notice the characteristic markings on the larger diseased plant—the whitish yellow sheath of the lowest affected leaf, the short narrow stripes at the base of the next leaf, and on the later leaves the whitening of the entire breadth at the base and the extension of broad stripes increasingly far into the normal green of each successive leaf tip. The leaves are nearly as broad and flexible as in normal plants, and their growth is little checked, if at all. The plants are 31 days old and developed the symptoms of the disease 25 days after emerging from the soil. One-seventh natural size.



PLATE 16¹

A.—Portion of a field of Moro White maize, showing heavy loss from the downy mildew. At the left, near the scale, is seen the only healthy plant that remains in this part of the field. Near it are several pale, stunted plants which are already withering, while in the background may be seen other plants less seriously attacked.

B.—View near the edge of a field of Guam White Dent maize, showing the ravages of the downy mildew. The tall, dark-leaved plant near the scale and two others a little farther back are the only healthy plants seen. Notice the stunted, withering specimens in the foreground, and at the right the seriously affected individual with stiffly ascending, striped leaves.

¹ On the scale which appears in this and the following photographs each black division equals 5 cm. Photographs by W. H. Weston.



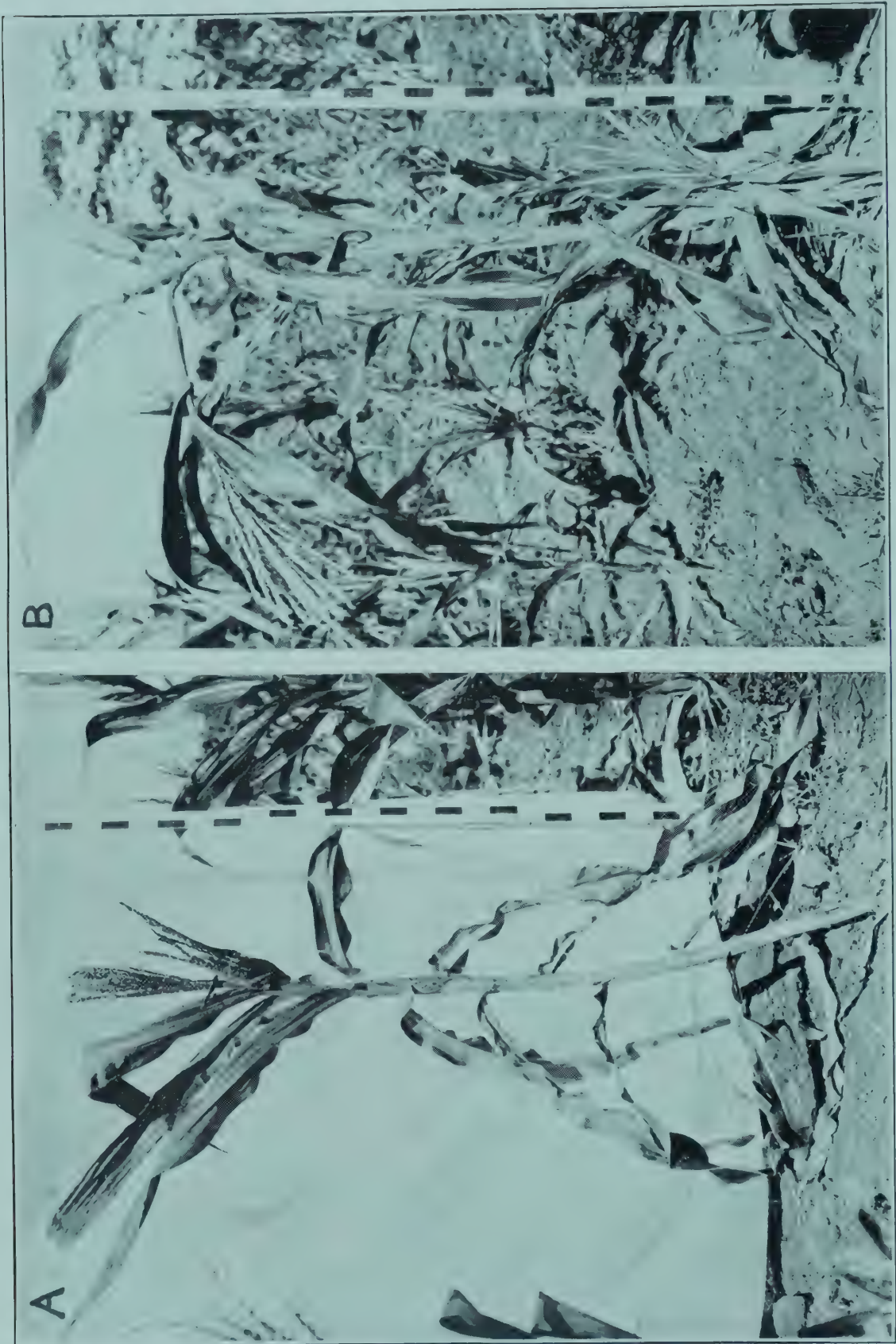


PLATE 17

A.—A frequently encountered type of downy mildew effect. The maize plant is sterile, with no ear borne in the normal place but with a couple of small, abortive ears growing at the base of the tassel. The leaf sheaths are whitish yellow, and conspicuous stripes of the same color occupy a large proportion of the leaves. These are stiff and brittle, the young ones at the top of the plant ascending at an unnatural angle and the older ones breaking and hanging down stiffly.

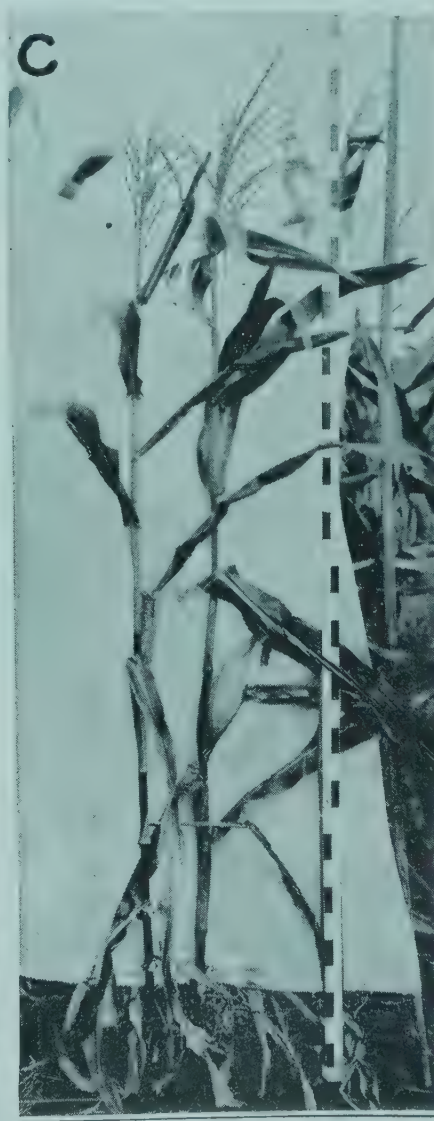
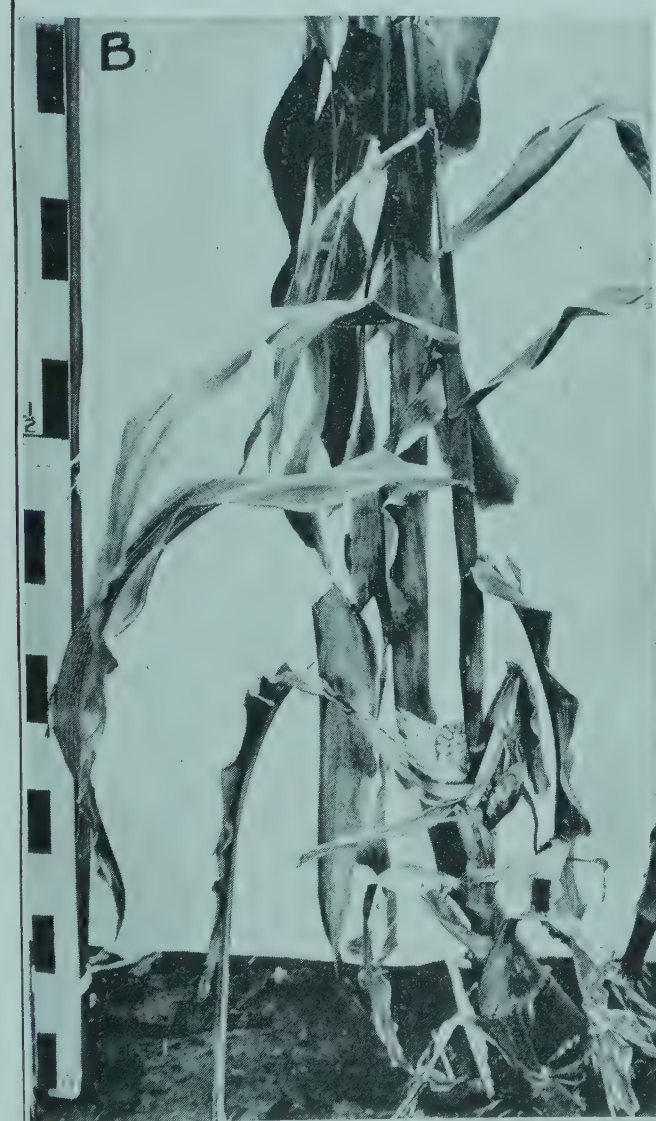
B.—A serious case of downy mildew injury, one of hundreds in a badly attacked maize field. The conspicuous striping of the leaves and the crooked stalk at once attract attention. The small ear is sterile. At the base of the plant can be seen another one badly dwarfed by the disease.

PLATE 18

A.—A common result of downy mildew attack. In both maize plants shown the growth of the internodes has been checked so that the leaf sheaths overlap and the unexpanded tassel is still partly surrounded by them. The striping of the leaves and their stiff, brittle character are easily seen. Both plants were entirely barren.

B.—A maize plant seriously injured by the downy mildew stands in front. Its stunted habit and striped leaves are striking evidences of the disease. Of the two abnormal ears, the one at the right was entirely sterile while the one from which the husks have been removed bore a few viable seeds. In the same hill, behind, is a healthy plant, only the lower part of which is shown.

C.—One hill in a maize plot which lost heavily from attacks by the downy mildew. The diseased plant at the left, although nearly as tall as the healthy companion at the right, is less strong and has a poorly developed ear which is only partly inclosed in husks and bears very few kernels.



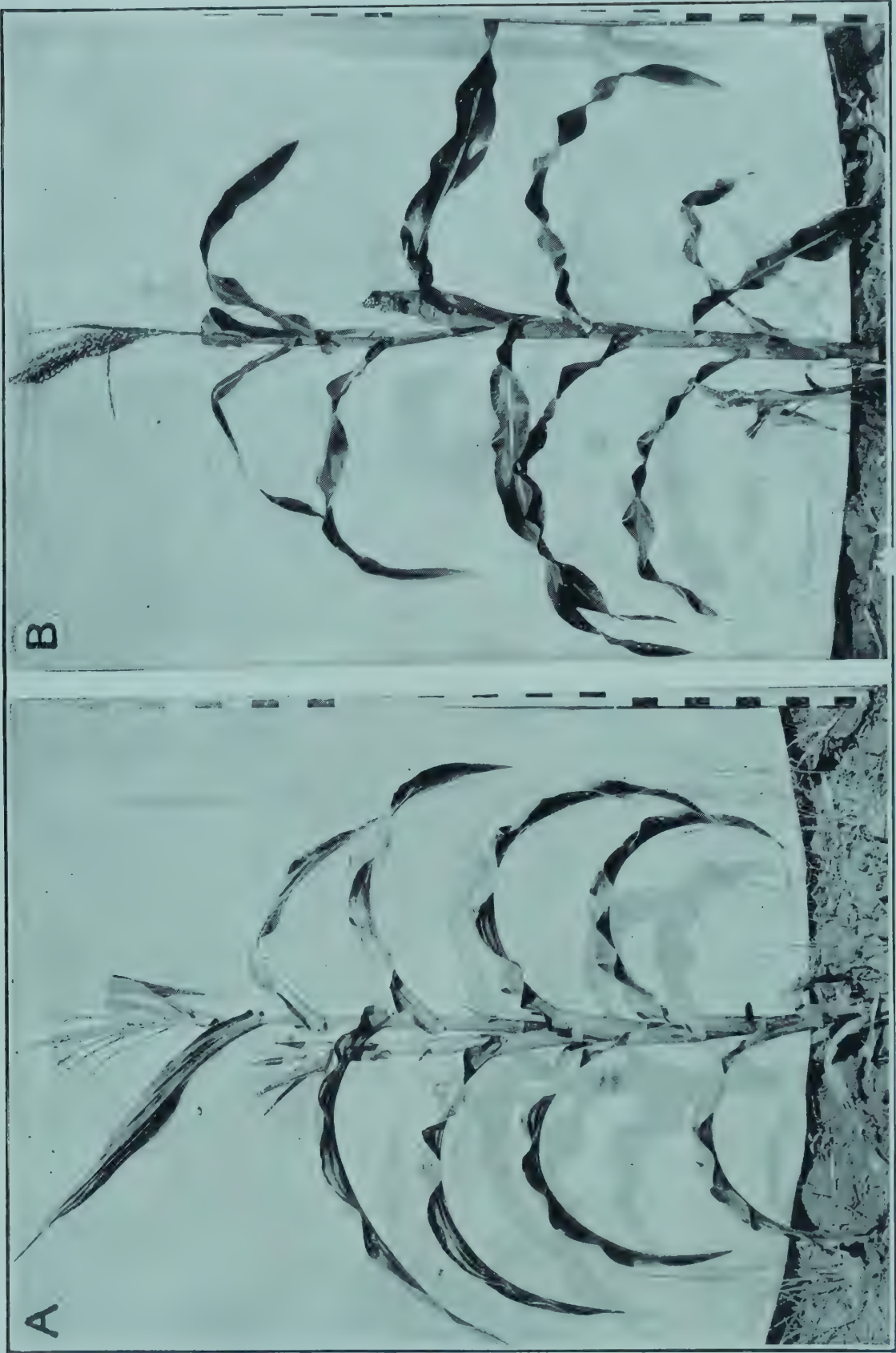


PLATE 19

A.—A case of abnormal growth of a maize plant as the result of an attack by downy mildew. The shank has elongated enormously, and an excessive development of the husks has taken place. Only a small, completely sterile ear has formed.

B.—A maize plant which, when nearly mature, became infected by the downy mildew through a small sucker previously developed. The sucker is obviously infected, but the large plant, aside from faint leaf stripings which escape the camera, shows the effect of the disease only in its unexpanded tassel and protruding ear tip.

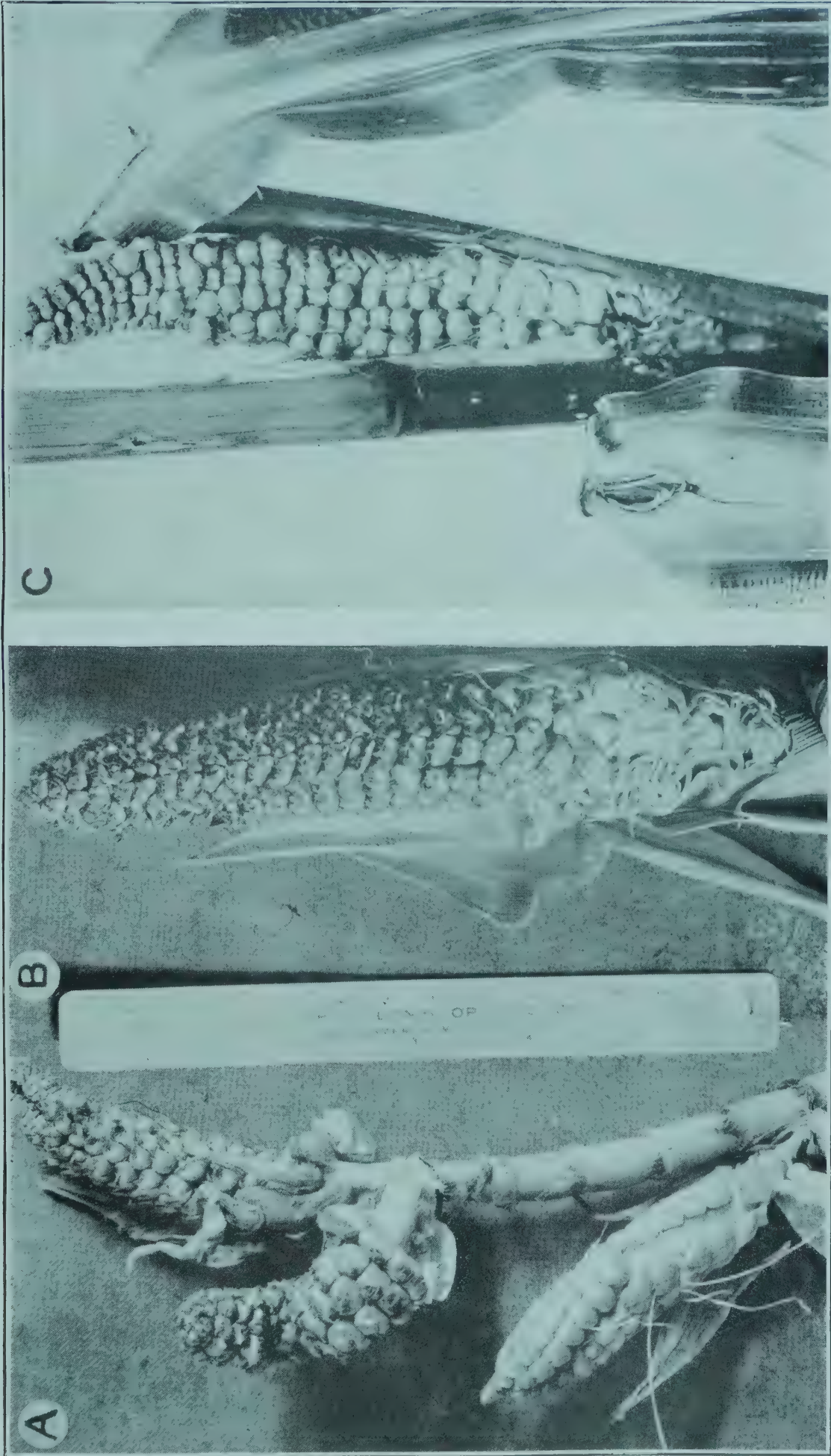
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PLATE 20

A.—A deformed and partly sterile ear complex produced by a maize plant as a result of downy mildew infection. Notice the branching and elongation of the shank, the abnormal development and arrangement of many of the kernels, and the inclosing of a few kernels in tunicate bracts. The husks have been removed. This specimen is from a Yellow Dent variety that normally has one large and well-developed ear.

B.—A maize ear developed abnormally as a result of the downy mildew. The husks, beyond which the upper third of the ear protruded, have been partly removed. Save for two or three at the base with partly developed kernels, all the florets were sterile, green in color, and bract-like in texture. Healthy plants of this Yellow Dent variety bear large ears well covered over by husks.

C.—Ear of a maize plant infected by the downy mildew. Only a few viable seeds have been formed, the remainder of the florets being poorly developed and sterile. Notice the conspicuous stripes on the leaves. Before the husks were removed the tip of the ear protruded beyond them. Normally this White Flint variety bears long, well-filled ears.



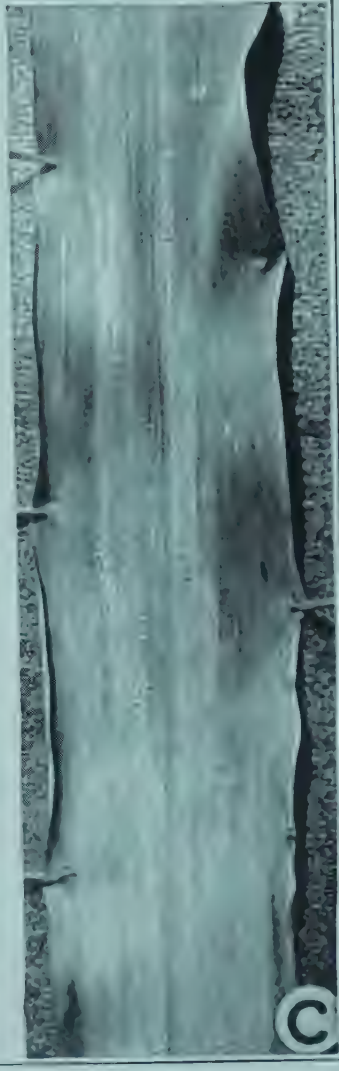
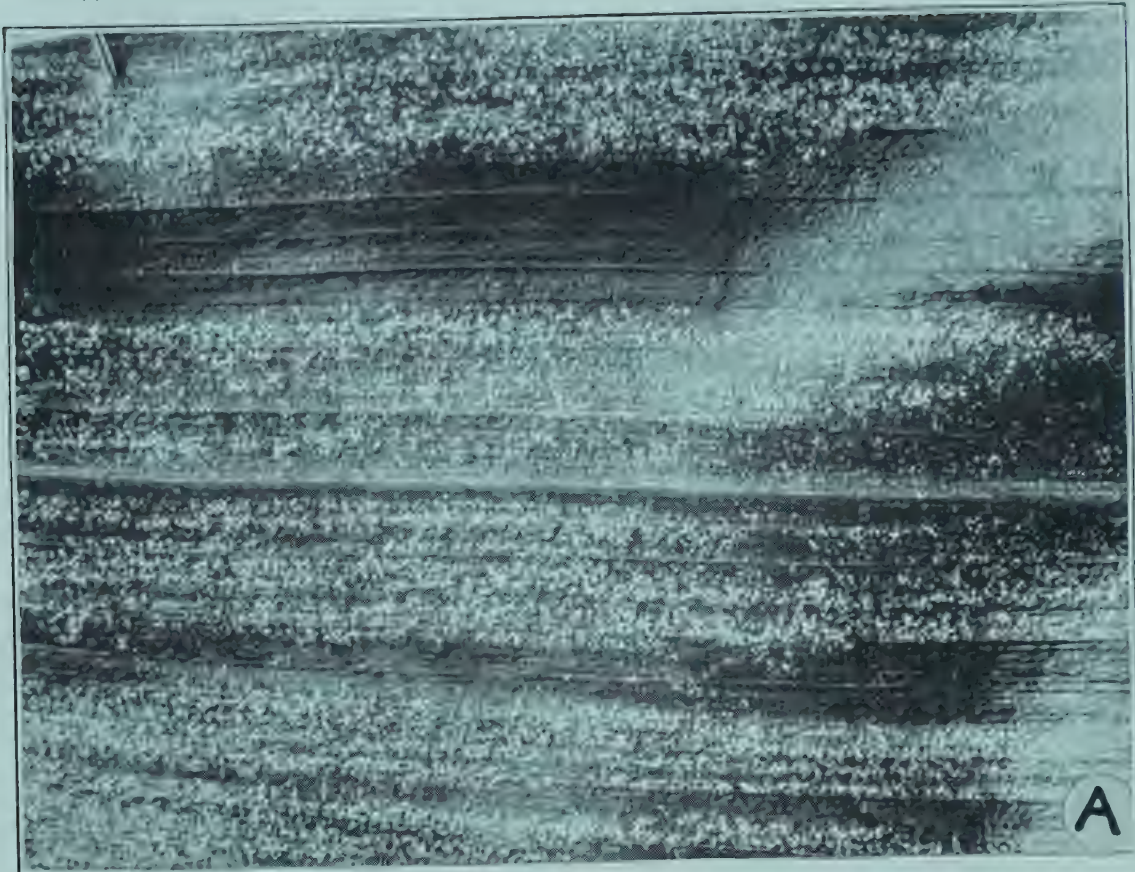


PLATE 21

A.—A near view of the thick down of conidiophores which has been produced on the upper surface of a badly diseased maize leaf. This gives an idea of the vast numbers of conidiophores which are borne on even a small area. The regions where they are formed most abundantly correspond in general to the stripings of the leaf. The layer of dew in which the conidiophores were produced has just dried. $\times 2$.

B.—Upper surface of a badly infected maize leaf from a maturing plant. Conidiophore production is in this case restricted to the yellowish white stripes like those shown in the colored plate. $\times 1\frac{1}{2}$.

C.—Upper surface of the middle portion of a maize leaf from a very young plant which has only recently developed the markings of the disease. The stripes are seen to be covered with conidiophores even up to the ends. $\times 1\frac{1}{2}$.

PLATE 22

A.—View of a row of Egyptian sorghum showing tall, green, healthy plants at the left and at the right a dwarfed, yellowish white plant which is infected by the downy mildew.

B.—Near view of this diseased sorghum plant. Notice the slender, stunted habit, the pallor and faint stripings of the leaves.

C.—A comparative view of healthy teosinte (right), and teosinte seriously infected with the downy mildew (left). The healthy plant has many suckers and is large and vigorous with broad, flexible, dark green leaves and well-developed inflorescences. The diseased plant has no suckers, is stunted and weak, and bears slender, stiff, brittle leaves, which are pale in color and inconspicuously striped, and poorly developed tassels containing a few abortive seeds at the base.

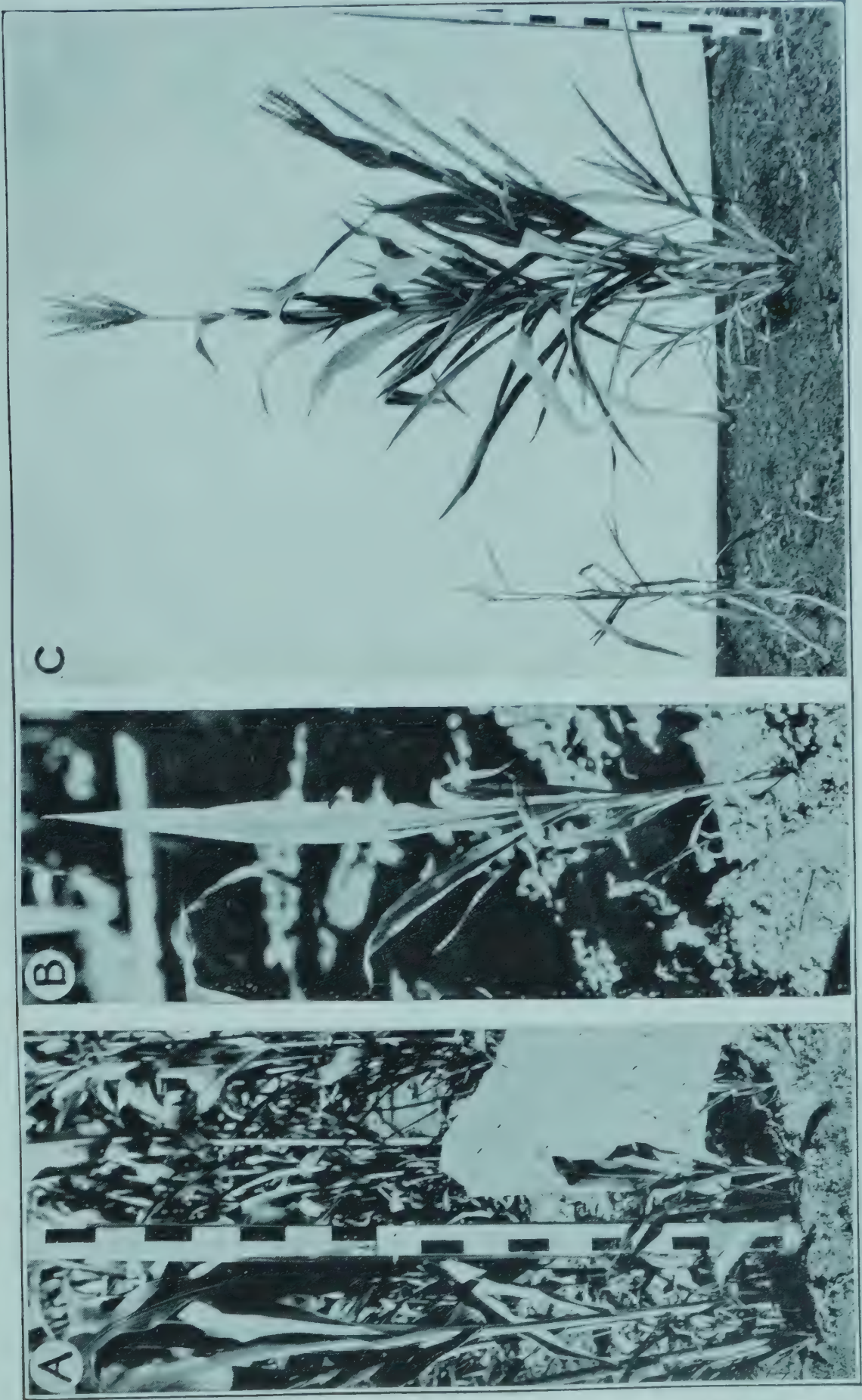




PLATE 23¹

A.—Portion of the typical crooked, irregular mycelium with numerous haustoria which is found in the mesophyll of badly infected leaves, here freed from the host tissue by maceration. $\times 375$.

B.—Longitudinal section cut from the center of a maize stem 8 inches from the ground. The plant, over 5 feet in height, was just putting out its tassel and had recently shown markings of the disease on its four uppermost leaves. A strand of the mycelium can be seen running alongside the bundle between cells of the bundle sheath which are penetrated by numerous haustoria. $\times 375$.

C.—Portion of the mycelium freed by maceration from tissue of the midrib at the base of a badly infected leaf. $\times 375$.

D.—Hypha cut in cross section as it lies between three adjacent mesophyll cells of the host. The penetration of a characteristic haustorium into one of the host cells is shown. $\times 850$.

E.—Transverse section from a badly infected portion of a maize leaf, showing the abundant mycelium running between the cells of the bundle sheath and forming in the substomatal air chamber the branches (*a*) that grow out through the stoma to form the conidiophores. The haustoria are seen penetrating not only the mesophyll cells but also a cell of the xylem (*b*) and the epidermis (*c*). $\times 375$.

F.—Portion of a hypha lying between adjacent mesophyll cells, one of which has formed a many-layered wall around the haustorium invading it. $\times 850$.

G.—Portion of a hypha similar to that shown in F but with the haustorium unhindered in its invasion of the host cell. $\times 850$.

H.—Bit of mycelium such as is shown in A but more highly magnified to show the haustoria. $\times 850$.

¹The drawings were made with the aid of a camera lucida and are all from preserved material of maize.

PLATE 24¹

A.—Slender, sparingly branched conidiophore bearing comparatively few conidia. It is only partially matured, as can be seen from the small size and rotund shape of the conidia and from the incomplete development of the septum. From maize during heavy dew. $\times 375$.

B.—Tip of branch with two conidia *in situ*. Treated with osmic acid and stained, thus differentiating the two sterigmata as more hyaline than the branch tip. $\times 750$.

C.—Stout, much-branched, mature conidiophore bearing 38 spores. From maize during heavy dew. $\times 375$.

D.—Upper portion of a nearly mature conidiophore with one secondary branch which has failed to branch further and has terminated in a single conidium only. $\times 375$.

E.—Small, stunted, sparingly branched conidiophore produced on maize during the light dew of the hot, dry season. Note the poorly formed cell and the small size and restricted development of the conidiophore as a whole in comparison with those formed in heavy dew, as shown in A and C. $\times 375$.

F.—Basal cell with two thick crosswalls; From maize. $\times 375$.

G.—An unusual basal cell with two septa and an abnormally large footlike base. $\times 375$.

H, J, L.—Typical basal cells of conidiophores. $\times 375$.

I.—Upper portion of an underdeveloped conidiophore bearing three spores on sterigmata arising directly from the top of the main axis. $\times 375$.

K.—Tip of an ultimate branch with two sterigmata bearing conidia. The right conidium is shown as if in optical section, the left in surface view. $\times 850$.

M.—Basal cell of a conidiophore from teosinte with septum formation progressing by the centripetal extension of a cellulose-pectose ring. The footlike projection at the base is abnormally large. $\times 375$.

¹The drawings were made with the aid of a camera lucida and are from fresh material, with the exception of B, G, K, and M. G, I, and M are from material on teosinte; all other figures are from material on maize.





PLATE 25¹

A.—Conidiophore from sorghum, partly matured and bearing few conidia. Compare with Plate 24, A. $\times 375$.

B.—Conidiophore from teosinte, nearly mature, with extensive system of branches bearing many conidia. Compare with Plate 24, C. $\times 375$.

C.—Typical conidia from sorghum. Three are germinating in dew by means of relatively simple hyphae. $\times 375$.

D.—Typical conidia from teosinte. $\times 375$.

E.—Typical conidia from teosinte which have germinated in dew on the leaf surface. $\times 375$.

F.—Conidium from teosinte germinating by an extensive branched hypha when maintained in dew at 7°C . $\times 375$.

G.—Conidium from teosinte germinating while still attached to its sterigma. $\times 500$.

H.—Typical conidia from maize, showing common variations in shape and size. $\times 375$.

I.—Two conidia from maize just beginning to germinate in rain water. $\times 375$.

J.—Two conidia from maize germinating in sterilized brook water maintained at 8°C . $\times 375$.

K.—Conidium from maize germinating in dew on the leaf surface. $\times 375$.

L.—Conidium from maize giving rise to extensive branching hyphae in a dilute decoction of young maize kernels. $\times 375$.

¹ The drawings were made with the aid of a camera lucida and are all from fresh material with the exception of A and G.



EFFECT OF DRUGS ON MILK AND FAT PRODUCTION

By FRANK A. HAYS, *Associate*, and MERTON G. THOMAS,

Assistant in Animal Husbandry, Delaware Agricultural Experiment Station

The opinion that milk production and butter-fat yield can be influenced by the use of drugs is widespread among dairymen. Many have their own opinions on this question, and some prominent feeders have been accused of "drugging" test cows. Three of our advanced registry associations now prohibit the use of all drugs during the official test period.

Marshall¹ states that some drugs and feeds are said to increase the milk and butter-fat yield. Friedberger and Fröhner² inform us that a number of galactogogues have always been recommended together with a liberal supply of feed largely fluid in character. They mention preparations of antimony, sulphur, chlorate of potash, fennel, juniper berries, caraway seed, aniseed, dill, and common salt. These writers recommend the "milk powder" used as drug No. 5 in the experiment reported below.

The value of an experimental test of different drugs lies not in the fact that it might make possible some abnormal test records in the hands of the unscrupulous but in the fact that it will furnish some information on the relation of feed components to the complex physiological processes of milk secretion.

PREVIOUS WORK

Henderson³ reports the effects of using six different drugs as galactogogues. Each drug was used on 10 cows, and the period of treatment was either two days or one week, with a control period of equal length either before or after treatment.

Henderson summarizes his results as follows:

1. With sodium bi-carbonate the cows increased the milk yield but neither the fat production nor the per cent. of fat in the milk.
2. With ginger the cows increased the per cent. of fat in the milk but decreased the milk yield and total fat production.
3. With pilocarpine hydrochlor injected into the cows hypodermically in most cases the cows increased both the per cent. of fat in the milk and total milk production.
4. With malt extract the cows in most cases appeared to increase the milk and butter fat production, but it had no effect upon the per cent. of fat in the milk.
5. Neither gentian nor powdered nux vomica had any effect either on the milk production or on the quality of the milk.
6. When grain alcohol was applied to the udder just previous to milking, no effect on the milk production or per cent. of fat in the milk was noted.

¹ MARSHALL, Francis H. A. *THE PHYSIOLOGY OF REPRODUCTION* . . . p. 566. London, 1910.

² FRIEDBERGER, FRANZ, and FRÖHNER, EUGEN. *VETERINARY PATHOLOGY*. Translated by M. H. Hayes. ed. 6, v. 1, p. 396-397. London, Chicago, 1908.

³ HENDERSON, Harry Oram. A STUDY OF FORCED FEEDING AND METHODS USED IN ADVANCED REGISTRY FEEDING. In *Penn. Agr. Exp. Sta. Ann. Rpt.* 1915/16, p. 393-419. 1918.

McCandlish¹ reports two series of trials with galactogoges. In the first series one cow was used and in the second there were three. The experimental period covered two days in each series, with a control period of two to four days following.

Results as given by McCandlish may be summarized as follows:

1. On the whole, alcohol depressed rather than stimulated milk and butter fat production.
2. Castor oil decreased the percentage of fat in milk, but the changes in milk yield were not appreciable.
3. Pitutarin treatment resulted in decreased milk and butter-fat yield.
4. Administration of pilocarpine and physostigmine resulted in an increased fat yield in the first series. One of the cows in the second series showed an increased fat yield, while the other two showed a decrease in milk yield.
5. The effect of aloes was greatly reduced milk yield and a fat yield somewhat reduced, but the averages show little change.
6. A mixture of epsom salts, common salt, and nux vomica showed only slight effect on milk and fat yield.

THE EXPERIMENT²

The experiment was begun April 14, 1919, and closed July 11, 1919. The objects of the experiments were:

1. To determine the effect of various drugs on the butter-fat test of milking cows.
2. To study the effect on the total fat yield of producing cows.
3. To determine whether drugs have an effect on the health or on total milk production.

METHOD

Four cows of mature age were chosen as experimental animals. No. 1 was a grade Holstein, No. 2 was a pure-bred Holstein, and No. 3 and 4 were pure-bred Guernseys. The interval of experimentation with each drug was five days. A control period of five days preceded all experimental periods, except the first five days of the experiment. Each of the four cows received a different drug for a 5-day period. This was followed by a 5-day control period during which no drugs were given. At the end of this period the drugs were shifted so that each cow received a different drug from the one previously given. Thus the control and experimental periods alternated, and the order in which the drugs were given was so arranged that each cow received each of the eight drugs for a 5-day period.

The cows experimented upon were milked twice daily, the weight of milk was recorded, and composite samples of the milk from each cow were tested for butter fat daily.

Drug mixture No. 1 was recommended to us by a prominent dairyman. The mixture No. 5 is one recommended by Friedberger and

¹ McCANDLISH, Andrew C. THE POSSIBILITY OF INCREASING MILK AND BUTTERFAT PRODUCTION BY THE ADMINISTRATION OF DRUGS. *In Jour. Dairy Sci.*, v. 1, no. 6, p. 475-486. 1918.

² Credit is due Dr. C. C. Palmer for administering drug No. 6 hypodermically.

Fröhner.¹ All the drugs except No. 6 were given mixed with the grain feed twice daily.

DRUGS USED

1. Food tonic consisting of 100 pounds oil meal, 5 pounds saltpeter, 5 pounds epsom salts, 5 pounds gentian, 5 pounds fenugreek, 8 pounds powdered charcoal, and 5 pounds sulphur, fed at the rate of 2 ounces daily per cow in two feeds.

2. Air-slaked lime, fed at the rate of 2 ounces daily per cow in two feeds.

3. Fowler's solution of arsenic, fed at the rate of 2 fluid ounces daily per cow in two feeds.

4. Gentian fed at the rate of 2 ounces daily per cow in two feeds.

5. Tonic mixture consisting of the following: 3 ounces black sulphid of antimony; 1½ ounces sulphur; 5 ounces each of fennel, caraway, and juniper berries, 1 pound common salt, fed at the rate of 2 ounces daily per cow in two feeds.

6. One gr. physotigmine sulphate injected hypodermically daily per cow, ½ grain in two doses.

7. Sodium bicarbonate, fed at the rate of 2 ounces daily per cow in two feeds.

8. Ginger, fed at the rate of 2 ounces daily per cow in two feeds.

EXPERIMENTAL RESULTS

Figures 1 to 8 present graphically the individual milk and butter-fat yield of each cow. A solid line is used for the control period and a dotted line for the experimental period.

Figure 1 shows the results of the tonic mixture. There was a slight increase in fat for the pure-bred Holstein and for one of the Guernseys, but the other cows showed no perceptible change. The milk yield was slightly increased for one Guernsey and slightly decreased for the other three cows.

Figure 2 shows that air-slaked lime increased the fat yield in two cases and the milk yield in two cases.

Figure 3 shows that when Fowler's solution of arsenic was used, two cows increased in fat production and three in milk production.

Figure 4 indicates that powdered gentian has a tendency to increase fat yield slightly but has little effect on milk production.

Figure 5 shows that the German tonic mixture did not increase either fat or milk production.

Figure 6 seems to indicate that physostigmine sulphate has a depressing effect on both milk and fat yield.

Figure 7 unfortunately shows the fat record for only three cows. There is no indication of any appreciable effect of sodium bicarbonate on production

¹ FRIEDBERGER, FRANZ, and FRÖHNER, Eugen. OP. CIT.

Figure 8 indicates that cows fed ginger begin to decline in production about the second or third day.

Table I gives a summary of results, showing the average of the four cows in total milk and total butter fat and the average test as obtained by dividing the fat yield by the milk yield given in the table.

TABLE I.—Effect of drugs on milk yield, fat test, and fat yield during 5-day period

Drugs used.	Average total milk.			Fat test.			Average total butter fat.		
	Control.	Treated.	Gain or loss.	Control.	Treated.	Gain or loss.	Control.	Treated.	Gain or loss.
	Pounds.	Pounds.	Pounds.	Per ct.	Per ct.	Per ct.	Pounds.	Pounds.	Pounds.
Food tonic No. 1.....	92.2	93.7	+ 1.5	4.37	4.57	+0.20	3.998	4.285	+0.287
Air-slaked lime.....	67.3	81.9	+14.6	5.21	5.00	— .21	3.509	4.095	+ .586
Fowler's solution of arsenic.....	107.3	109.0	+ 1.7	3.72	3.86	+ .14	3.995	4.210	+ .215
Gentian.....	113.5	108.2	— 5.3	3.93	3.98	+ .05	4.459	4.302	— .157
German tonic mixture...	108.8	108.0	— .8	3.97	3.75	— .22	4.205	4.049	— .156
Physostigmine sulphate...	108.7	99.9	— 8.8	3.89	3.60	— .29	4.226	3.706	— .520
Sodium bicarbonate.....	93.1	90.8	+ 2.3	4.43	4.43	+ .00	4.132	4.020	— .112
Ginger.....	92.5	93.8	+ 1.3	4.28	4.32	+ .04	3.968	4.055	+ .087

Drugs 1, 2, 3; and 8 slightly increased the milk yield, but this increase is insignificant except when air-slaked lime was fed. The increase of 21.7 per cent for the air-slaked lime group we think is significant. Gentian and physostigmine sulphate seem to depress the milk yield, and the German tonic mixture No. 5, and sodium bicarbonate have but little effect.

The fat test was appreciably increased by tonic No. 1, by lime, and by Fowler's solution. There was significant decline in fat test shown by the groups fed the German tonic mixture and physostigmine sulphate.

Average total butter-fat production was probably significantly increased by air-slaked lime. Food tonic No. 1 and Fowler's solution show increase of 0.28 and 0.21 pound, respectively, in fat for the 5-day period. A decline of 0.52 pound is shown by the physostigmine sulphate group. The decline in other groups is not considered significant.

No difficulty was encountered in getting the cows to take any of the drugs, and no effect on their physical condition was observed.

SUMMARY

(1) A study of individual records and average records does not indicate that drugs have a very pronounced effect on the production of the dairy cow.

(2) Air-slaked lime fed in 2-ounce doses daily may possibly increase milk production and total fat yield.

(3) No other drug or mixture tested proved to be of value to increase production.

(4) Results do not indicate that the difference in character of milk of Holstein and Guernsey cows has any relation to their manner of reaction to drugs.

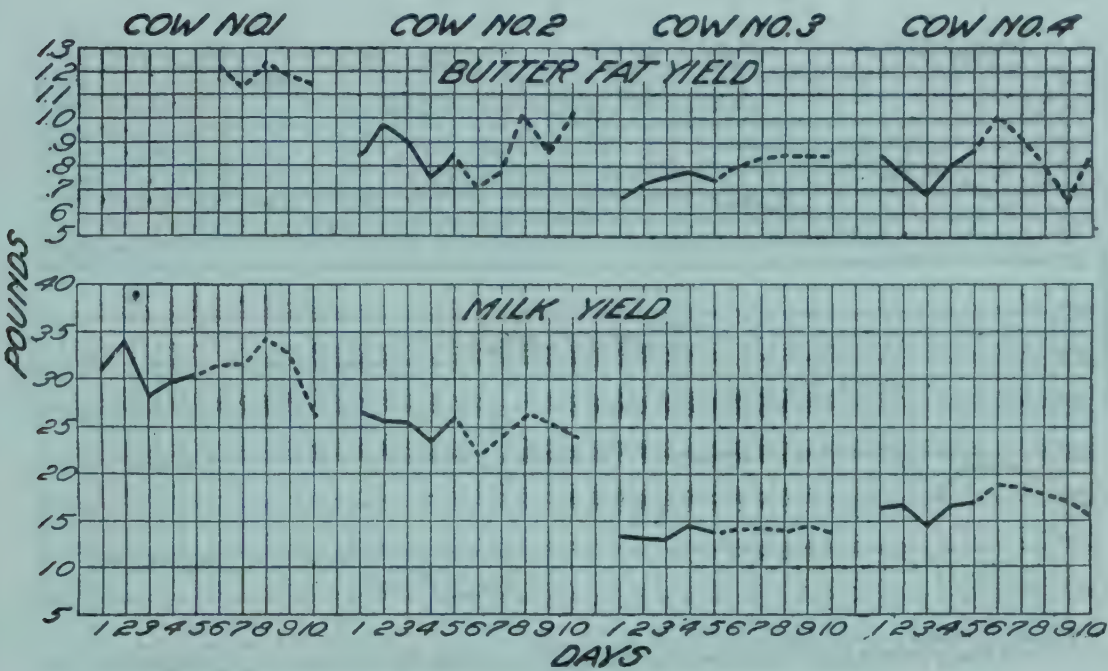


FIG. 1.—Graph showing effect of tonic mixture No. 1 on butter-fat and milk yield.

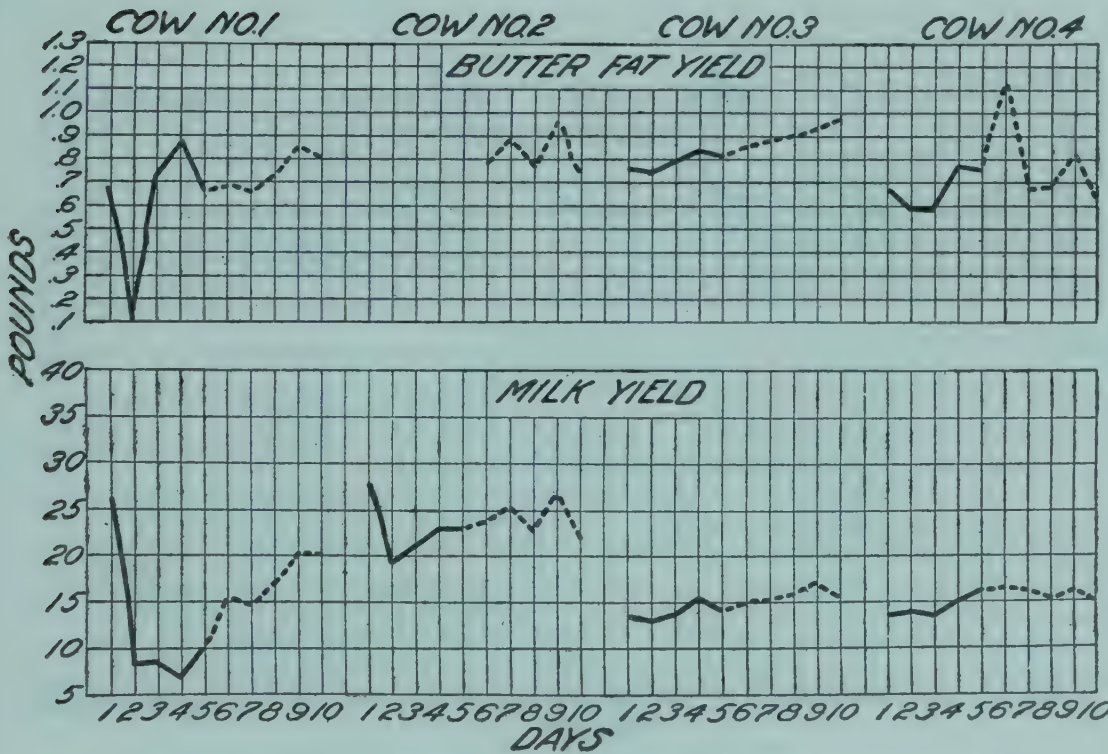


FIG. 2.—Graph showing effect of air-slaked lime on butter-fat and milk yield.

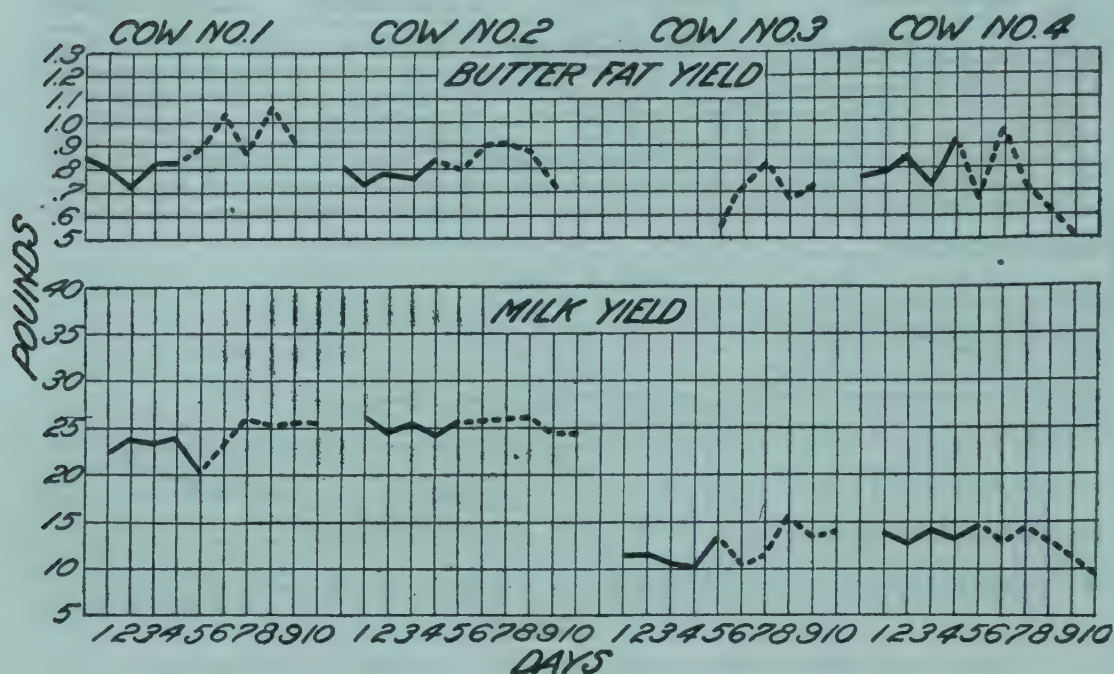


FIG. 3.—Graph showing effect of Fowler's solution of arsenic on butter-fat and milk yield.

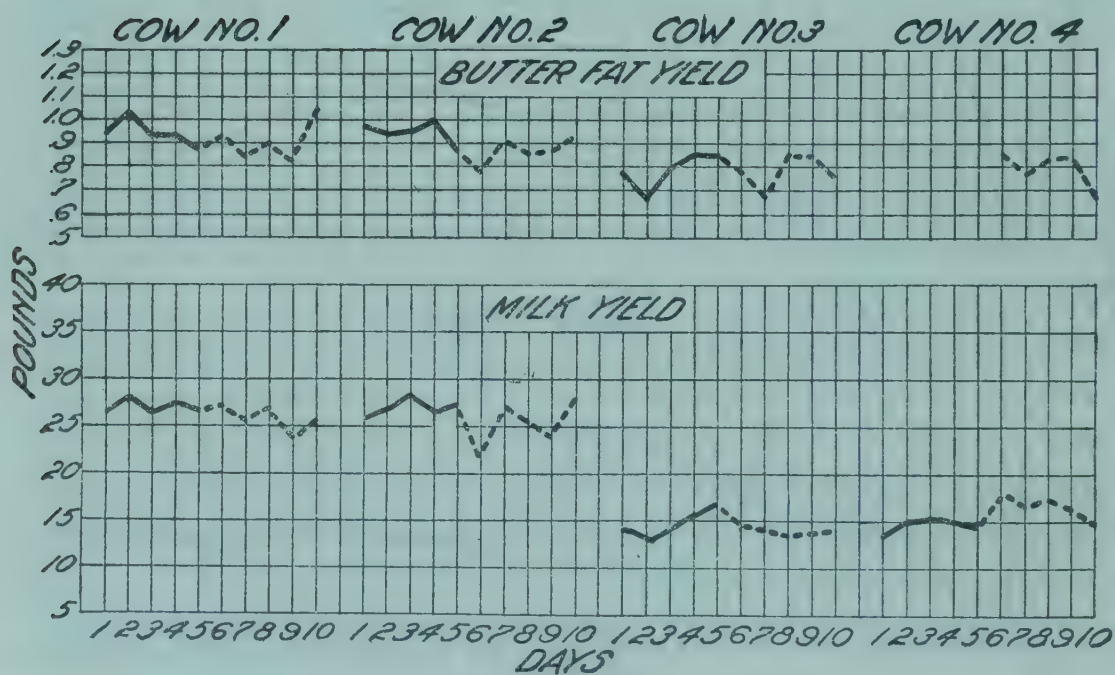


FIG. 4.—Graph showing effect of powdered gentian on butter-fat and milk yield.

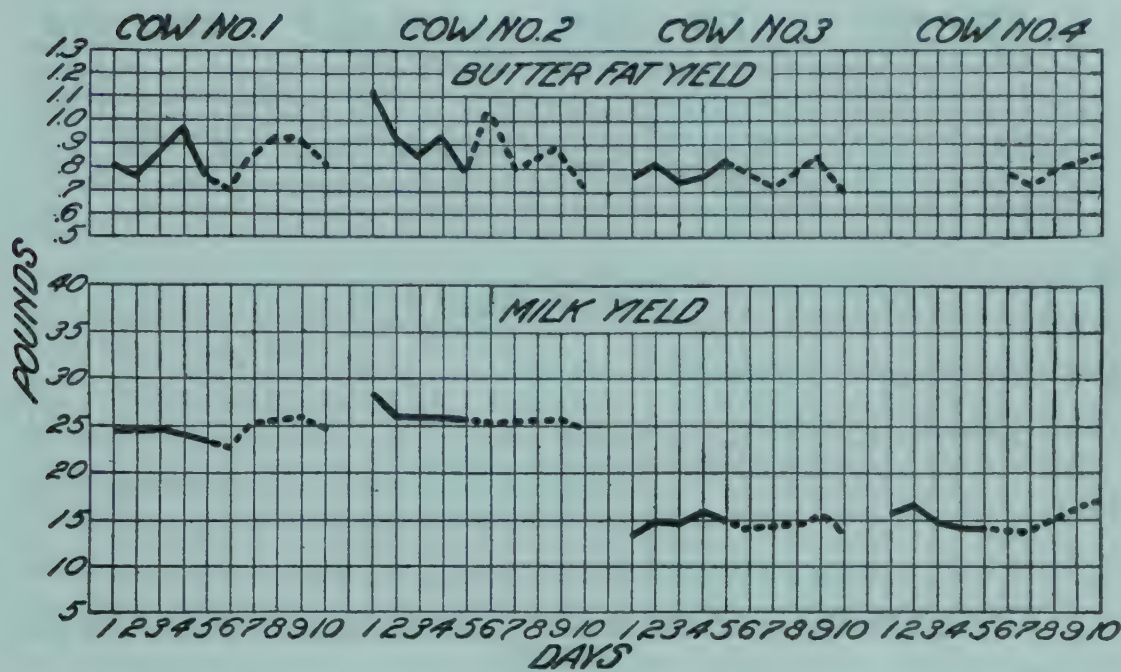


FIG. 5.—Graph showing effect of the German tonic mixture on butter-fat and milk yield.

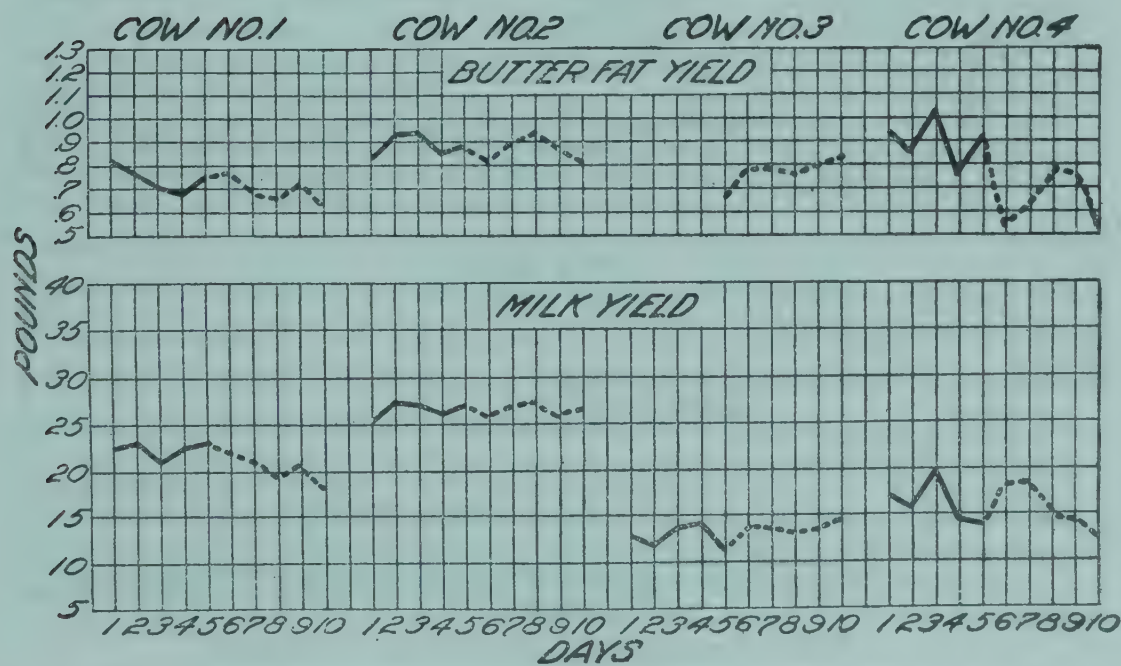


FIG. 6.—Graph showing effect of physostigmine sulphate on butter-fat and milk yield.

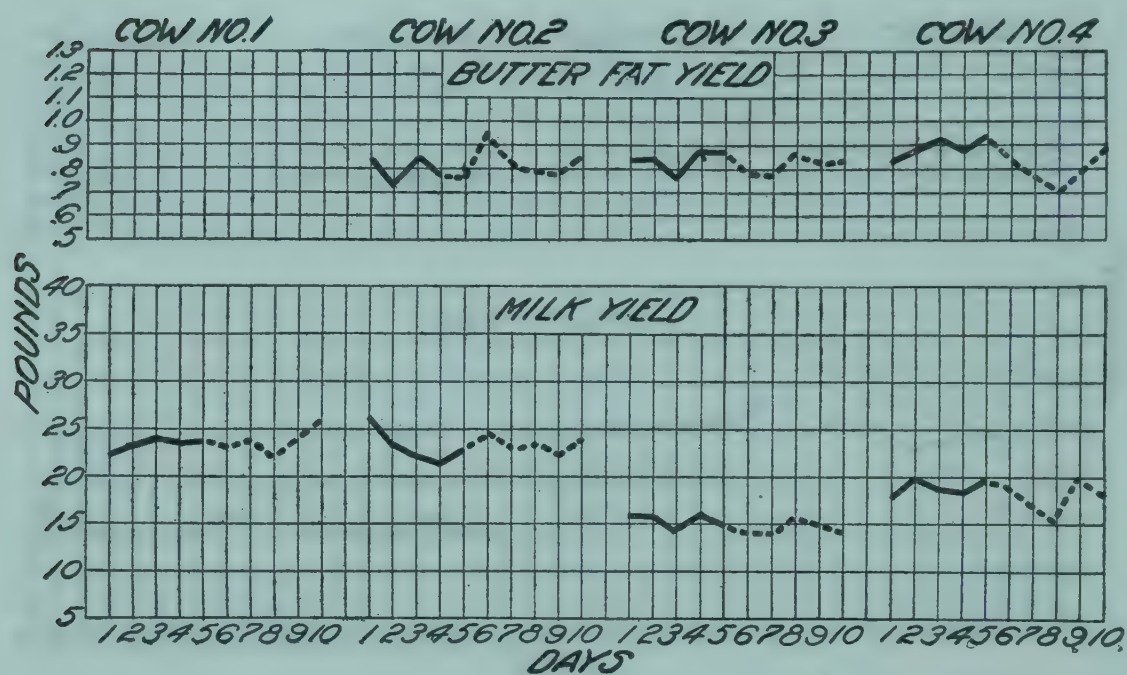


FIG. 7.—Graph showing effect of sodium bicarbonate on butter-fat and milk yield.

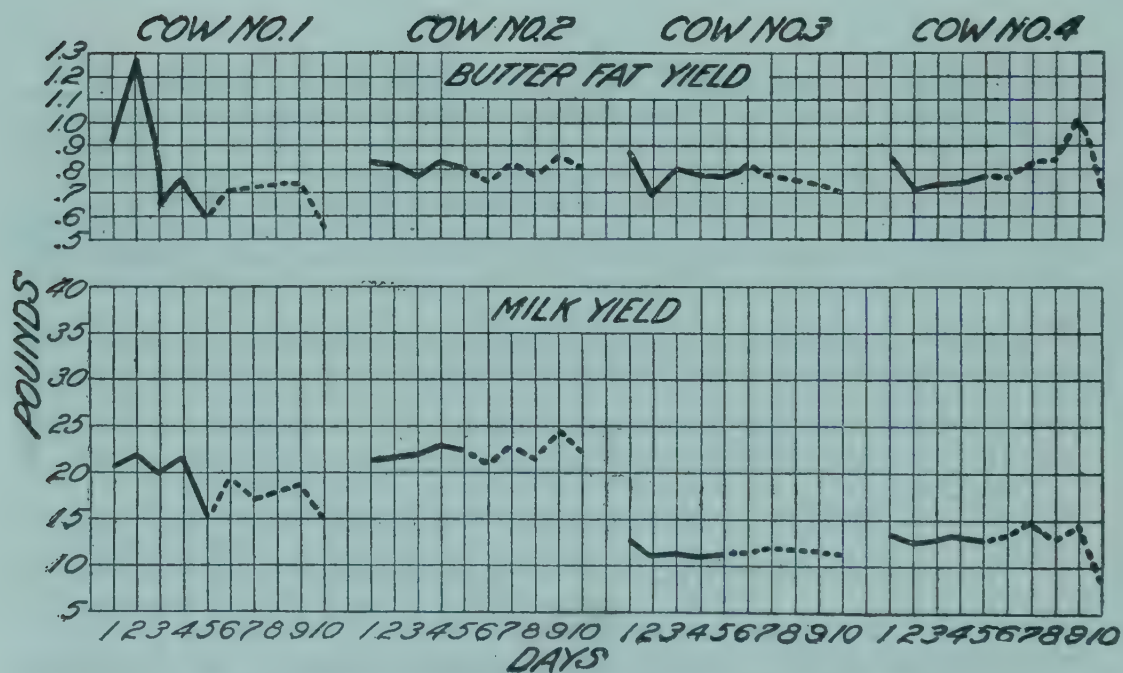


FIG. 8.—Graph showing effect of ginger on butter-fat and milk yield.

ARTIFICIAL AND INSECT TRANSMISSION OF SUGAR-CANE MOSAIC

By E. W. BRANDES

Pathologist, Office of Sugar-Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture

The infectious nature of sugar-cane mosaic can hardly be questioned in the light of field observations bearing out this point made in Georgia and Florida last year and in Porto Rico during the preceding two years (1)¹. Records of well-controlled inoculation experiments, however, have been wholly lacking. A number of investigators, beginning with the Dutch workers in Java, have attempted to produce the disease by artificial inoculation and by the use of suspected insect carriers; but in all cases results have been negative or inconclusive. Where success has been reported the experiments were carried on under unsatisfactory conditions, and the results were repudiated by contemporaneous workers who attempted to repeat the experiments. Kamerling (3) in 1902 reports that he secured infection by inoculating healthy plants with sap from diseased plants. He says (in translation):

So far as the kind of disease is concerned, we are dealing with a disease analogous to the notorious mosaic of tobacco, that is, with an infectious disease, which, however, in all probability is not caused by a parasitic organism.

As is the case with tobacco mosaic, the disease has been successfully transmitted by inoculating healthy plants with juice pressed out of diseased plants. (Footnote: My inoculations with juice of diseased cane were performed in the same way as the inoculation tests of Beijerinck with juice of tobacco plants affected with mosaic.)

These inoculation tests, however, throw little light on the manner of origin and of dissemination in nature.

One very great difficulty in carrying out tests on the way in which the disease originates and is disseminated in nature is in securing cuttings that do not have a predisposition toward the disease. From the best possible selected Moga cuttings a certain number of check plants in my pot cultures showed stripe disease; and I have had a similar experience with specially selected cuttings from Van Delden in Soekaboemi, which in Koeningen produced a crop practically free from disease.

This vague reference to his experiments and his admission of disease in the control plants was not very convincing and was discredited by later Dutch investigators. Kobus (4), van der Stok (6), and Wilbrink and Ledebøer (7) were unable to produce the disease by using the method of Kamerling. Wilbrink and Ledebøer say (in translation):

So sudden a severe outbreak as Kobus already observed gives rise to the suspicion that we are perhaps dealing with an infectious disease, as is the case, for example, with the mosaic disease of tobacco, analogous to the stripe disease in very many respects. Dr. Kamerling states in the Annual Report of the Experiment Station of Kagok for

¹ Reference is made by number (italic) to "Literature cited," p. 138.

1902 that he succeeded in inoculating healthy plants with the disease by injecting sap from diseased plants. We have repeated these inoculation experiments as far as we have been able to obtain data about them, but without success. Neither have we been able to find any other indication that the disease is contagious.

They conclude with Kobus and van der Stok that the mosaic is an expression of bud variation. No reference is made to successful inoculation experiments in the numerous papers on mosaic in the Hawaiian Sugar Planters' Record for 1911-1919. Stevenson (5) reports hundreds of inoculations of many cane varieties by various methods during 1917 and 1918, all with negative results. Prof. F. S. Earle, in an unpublished paper, calls attention to a method of inoculating with juice expressed under oil to prevent oxidation. Some of the plants he inoculated became diseased, but the experiment was inconclusive and open to the criticism that it was carried on without control plants in a field where cases of the disease were appearing naturally.

Various writers have called attention to the possibility of insect carriers of the mosaic disease, but no published proof has appeared, and the statements have been based on analogy with other apparently similar diseases and on field observations. The failure of all efforts to obtain uniform or dependable results with either artificial methods of inoculation or with insects has been one of the conspicuous peculiarities in the behavior of sugar-cane mosaic. In all inoculation work in plant pathology it is necessary to secure a very high percentage of infection in inoculated plants where control plants are not absolutely protected from extraneous infection. In diseases like cane mosaic, where, for reasons which we are not in a position to discuss at present, the percentage of infection resulting from experimental inoculation is not high, it is not only necessary that all experimental plants be apparently healthy but also that they be of known healthy parentage for at least one generation back and preferably more. Further than this, the experiments should be performed under absolutely controlled conditions. The prevention of contamination of experimental plants with diseased material by direct or indirect contact must be absolute. Special precautions must be taken to prevent the admittance to treated plants of insects or any other animals other than the ones being experimented with.

The writer became convinced, after observations and experiments with the mosaic disease dating from the summer of 1916, that more reliance can be placed on the results of experiments performed in some region far removed from any chance of accidental infection. It was owing to these considerations that the experiments recorded here were performed at a distance from the seat of any natural infection, because the required conditions would be practically impossible to obtain where the disease is prevalent.

The first experiments were conducted in a quarantine greenhouse near Garrett Park, Md. Later experiments were made in several green-

houses at Washington. The insects used were those at hand which were known to feed on sugar cane. Provision has been made by cooperation with the Bureau of Entomology to collect information leading to the identification of the particular insect or insects responsible for secondary infections in the infested cane regions. Mr. George N. Wolcott, of the Bureau of Entomology, is at present working on that phase of the problem in Porto Rico.

EXPERIMENTS AT GARRETT PARK, MD.¹

Seed pieces from diseased parent stock were received from time to time during 1918 and 1919 and planted in the greenhouse, which was screened with physician's cloth so that insects could not escape. On August 10, 1918, a shipment of diseased Crystalina cane from Ensenada, P. R., was planted. Yellow Bantam sweetcorn and Sugar Drip, Early Amber, and Japanese Ribbon sorghum were planted August 13, 1918, in the same greenhouse. On September 24, 1918, a shipment of diseased Rayada cane from Rio Piedras, P. R., was planted. Diseased seed pieces of Morado, Yellow Caledonia, Crystalina, and Rayada varieties from Arecibo, P. R., were planted on April 24, 1919. Similar pieces of Selangore, D.-117, and Rayada from Mayaguez, P. R., were planted on April 25, 1919. Lastly a shipment from Yauco, P. R., containing diseased seed pieces of G. C.-701, G. C.-1486, B.-3922, B.-6450, and P. R.-260 were planted May 1, 1919.

Through the kindness of Dr. Erwin F. Smith, cuttings of Lahaina cane were secured from plants which had been growing in one of his greenhouses at Washington for more than six years and showed absolutely no signs of mosaic. These cuttings were planted in pots in a third greenhouse at Washington on December 10, 1918. All the cane, diseased and healthy, sprouted and grew well. All cuttings from diseased parents produced mottled sprouts, without exception, and all cuttings from Dr. Smith's healthy cane produced in great contrast healthy plants with leaves of uniform dark green color.

EXPERIMENT 1.—This was a preliminary experiment to determine whether infection could take place by natural means, merely by exposing healthy plants in the same greenhouse with diseased plants. On May 10, 1919, 5 healthy cane plants, 5 months old, in pots were taken from the greenhouse in Washington and placed in the quarantine greenhouse at Garrett Park, Md., in such a way that the leaves did not come in contact with the leaves of diseased plants. At that time the corn aphid (*Aphis maidis*)² was abundant on the sorghum. The wild grasses, a few clumps of which came up as weeds in the greenhouse, were infested with red spiders (*Tetranychus binaculatus*). Both these insects were seen occasionally in the cane. A small leafhopper was also seen but was not captured

¹ Thanks are due Dr. Caroline Rumbold, who was in charge of this work during the writer's absence on trips to the Tropics.

² Identified by Dr. A. C. Baker, of the Bureau of Entomology, United States Department of Agriculture.

and consequently was not determined. On June 3, 1919, all five of the Lahaina cane plants from Dr. Smith's greenhouse showed unmistakable incipient signs of mosaic. Two weeks later all were well-developed cases.

EXPERIMENT 2.—On July 3, 1919, 15 healthy cane plants of the Lahaina variety, 7 months old, were removed from the greenhouse in Washington to the "pesthouse" at Garrett Park. Five were placed within the house unprotected as before, and 5 were placed in each of two insect-proof cages. On July 22, 4 of the exposed plants showed incipient signs of mosaic. On August 2 the remaining plant showed evidence of being infected, and a week later all the exposed plants exhibited well-advanced leaf symptoms. At this time the 10 control plants in cages were perfectly normal and continued so until they were used in another experiment two months later.

EXPERIMENT 3.—Seeds of sweetcorn (Yellow Bantam variety) and sorghum (Sugar Drip, Early Amber, and Japanese Ribbon) were planted on August 13, 1918, in the Garrett Park quarantine greenhouse. They germinated and grew slowly during the winter, then more rapidly in the spring. A number of volunteer grasses that came up as weeds in the greenhouse were allowed to mature. All these plants soon became heavily infested with corn aphids. Sorghum seed from the same lot was planted in a greenhouse at Washington. On May 7, 1919, a few mottled leaves appeared on the sorghum plants at Garrett Park. Examination of the wild grasses revealed the typical streaking and mottling in practically every stool of crabgrass (*Syntherisma sanguinalis*), foxtail (*Chaetochloa lutescens*) and *Panicum dichotomiflorum*. Other wild grasses in the greenhouse were normal. At this time the sorghum control plants in the Washington greenhouse and the wild grasses of the same species outside the greenhouse at Garrett Park showed no signs of mosaic, nor did they show any evidence of mosaic during the remainder of the summer.

EXPERIMENT 4.—On August 7, 1919, about 50 adult individuals of the sharp-headed grain leafhopper (*Draeculacephala molipes*)¹ collected two days previously on mosaic-diseased sugar cane at Audubon Park, New Orleans, La., were placed in a cage with 5 healthy cane plants at the Garrett Park greenhouse. The leafhoppers immediately began feeding on the healthy cane. No infection was evident after two months.

EXPERIMENTS AT WASHINGTON

During September, 1919, nearly all experiments were transferred to greenhouses especially prepared to receive them at Washington. Ventilators of the 2-story greenhouse, formerly used by Dr. Smith for bananas, were screened with physician's cloth; and the diseased cane plants of all varieties were removed to it from Garrett Park. A greenhouse in another range, separated by a roadway from the first, was screened; and

¹ Identified by Mr. T. E. Holloway, Bureau of Entomology, United States Department of Agriculture.

300 healthy Lahaina cane plants, from cuttings supplied by Dr. Smith, were placed therein. These plants were from the same source as the ones previously mentioned. The second greenhouse was divided into halves by a tight glass partition. One compartment was used for propagating healthy stock, and the other compartment was used for artificial inoculation experiments. Both compartments were kept free from insects by frequent fumigation. In the banana house, or "pesthouse" fumigation was not practiced on account of cage experiments with insects. The greatest precautions were taken to prevent accidental infection of plants in the house where healthy stock was growing. This house was invariably the first one visited by the gardener for routine work such as watering, and both houses were kept padlocked at all times. Probably because of this care no single case of mosaic has appeared there or on control plants in either house in any of the experiments.

INOCULATIONS WITH INSECTS

EXPERIMENT 1.—On October 8, 1919, 10 individuals of *Aphis maidis* were transferred with a camel's-hair brush from mosaic sorghum to each of four young healthy cane plants in separate cages. A fifth cage was reserved for two healthy plants as controls. On October 28 all four plants showed incipient signs of mosaic. On November 18 they were all unmistakable, well-advanced cases. The two control plants remained healthy.

EXPERIMENT 2.—On February 2, 1920, 12 to 15 individuals of *Aphis maidis* were lifted from mosaic sorghum and placed on each of three healthy cane plants in separate cages. Two healthy cane plants were placed in a fourth cage for controls. On February 28 two of the treated plants showed signs of mosaic and on March 5 were typical cases. The two control plants remained healthy.

EXPERIMENT 3.—On February 2, 1920, one mosaic sorghum plant infested with *Aphis maidis* was placed in a cage with a healthy cane plant in such a way that the leaves of the two plants intermingled. On March 21 the cane plant showed unmistakable signs of infection.

EXPERIMENT 4.—February 2, 1920, 10 individuals of *Aphis maidis* were lifted from a diseased cane plant of variety G. C.-701 and placed on a healthy cane plant in a cage. No infection was apparent on March 15.

EXPERIMENT 5.—On October 8, 1919, 15 specimens of *Draeculacephala molipes* were placed in each of five cages containing one healthy and one mosaic cane plants. On January 5, 1920, approximately three months later, there was no evidence of infection.

EXPERIMENT 6.—On January 5, 1920, 15 specimens of *Draeculacephala molipes* were placed in each of two cages containing two mosaic sorghum plants and two healthy cane plants. On March 11 there was no sign of infection on any of the cane plants.

EXPERIMENT 7.—On November 20, 1919, two mosaic cane plants of the Rayada variety, infested with the sugar-cane mealy bug (*Pseudococcus boninensis* (Kuw.),¹ were placed in each of two cages, together with two healthy cane plants of the Lahaina variety. A few of the mealy bugs were transferred from diseased plants to all healthy plants. Ants were assiduously tending the mealy bugs. On March 11, 1920, all healthy plants were badly infested with mealy bugs but there was no mosaic infection.

ARTIFICIAL INOCULATIONS

Virus was obtained for artificial inoculation by two methods. Cell sap from young leaves, designated as virus No. 1, was obtained by grinding the young, tightly rolled leaves of diseased Rayada cane in a food chopper and straining through several thicknesses of cheesecloth. It was used undiluted for inoculating immediately after being prepared. Virus No. 2 consisted of cane juice from the youngest joints, including the growing point. To prevent oxidation this was pressed out under a mineral oil (Nujol) in a specially designed press (2). This also was used undiluted as soon as it was prepared. Inoculations were made in the compartment of the fumigated greenhouse separated from all diseased material and protected by every means from accidental infection. The results of these inoculations are given in Tables I and II.

In addition to the control plants injured with a sterile needle, there were about 100 other healthy plants of the Lahaina variety in the compartment. No case of mosaic developed among these plants.

TABLE I.—*Effect of artificial inoculation of Lahaina cane with triturated young leaves (virus No. 1)*

[Plants inoculated Jan. 8, 1920]

Number of plants.	Treatment.	Results.
10.....	Virus rubbed on unbroken surface of young leaves with fingers.	All healthy Mar. 21.
10.....	Youngest leaves inoculated by numerous needle pricks.	One mosaic Mar. 21.
5.....	Control plants pricked with sterile needle.....	All healthy Mar. 21.
10.....	Epidermal layer of young leaf cells scarified with sharp needle dipped in virus.	Do.
5.....	Control plants scarified with sterile needle.....	Do.
10.....	Young leaves scarified as above and virus rubbed in vigorously with the fingers.	Do.
10.....	Inoculated by injecting $\frac{1}{2}$ cc. of virus into growing point with hypodermic syringe.	Two mosaic Feb. 14; eight healthy Mar. 21.
5.....	Control plants punctured at growing point with sterile needle.	All healthy Mar. 21.

¹ Identified by Mr. Harold Morrison, of the Bureau of Entomology, United States Department of Agriculture.

TABLE II.—Effect of artificial inoculation of Lahaina cane with juice from cane unoxidized (virus No. 2)

[Plants inoculated Jan. 7, 1920]

Number of plants.	Treatment.	Results.
10.....	Virus rubbed on unbroken surface of young leaves with fingers.	All healthy Mar. 21.
10.....	Youngest leaves inoculated by numerous needle pricks.	Do.
5.....	Control plants pricked with sterile needle.....	Do.
10.....	Epidermal layer of young leaf cells scarified with sharp needle dipped in virus.	Do.
5.....	Control plants scarified with sterile needle.....	Do.
10.....	Young leaves scarified as above and virus rubbed in vigorously with fingers.	Do.
10.....	Inoculated by injecting ½ cc. of virus into growing point with hypodermic syringe.	Eight mosaic Feb. 6 to 14.
5.....	Control plants punctured at growing point with sterile needle.	All healthy Mar. 21.

DISCUSSION

From the foregoing results it may be inferred that the sugar-cane mosaic virus is highly infectious only when exacting demands in the matter of favorable conditions are satisfied. Erratic spreading under natural conditions in the field also indicates the necessity for special conditions, which are not as yet known. It is considered as proved, however, that the cell sap of diseased plants is infectious when introduced in the proper manner and that the disease can be transmitted by insects. Just what insects are responsible for dissemination in the cane regions remains to be proved. The failure of the sharp-headed grain leafhopper to transmit the disease under the conditions of these experiments is surprising. This insect is very common on cane in Louisiana, and as a result of field observations suspicion was directed toward it from the first. Other leafhoppers are now being tested. The successful experiments with the corn aphid is of great interest scientifically, but it is not believed that transmission of mosaic is restricted to this insect or to other aphids more abundant on cane. *Aphis maidis*, however, has been reported on sugar cane from practically every sugar-cane region in the world.

That cane mosaic is analogous with other mosaic diseases is brought out by a number of facts, aside from the visible signs of the disease. As in many other mosaics, the infectious material does not seem to be highly specialized, but may attack other plants of the same family. The cell sap of infected plants contains some organism, not visible by ordinary means, which is capable of inducing the disease when injected into healthy plants. Leaves which are mature at the time of inoculation never show any signs of mosaic. This fact, typical of all mosaics, has been brought

out in all inoculation experiments with sugar cane. The disease can be transmitted by certain sucking insects. There is no known period of saprogenesis in the existence of the virus. Seed transmission of the virus is one of the phenomena concerning which divergent results have been recorded for the various mosaic diseases. This point has not been definitely settled for sugar-cane mosaic, but mosaic sorghum plants failed to produce mosaic progeny in two experiments.

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HALO-BLIGHT OF OATS¹

By CHARLOTTE ELLIOTT

*Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry,
United States Department of Agriculture*

INTRODUCTION

The present paper is a description and discussion of a bacterial disease of oats which has been the subject of investigation by the writer for the past three years. This "halo-blight" is a disease which occurs to at least some extent each year throughout the oat-growing sections of the central and eastern States and becomes of economic importance during certain seasons when weather conditions are particularly favorable to its development.

During the season of 1918 field observations and specimens of diseased plants from widely separated sections of Wisconsin showed that this disease occurred in practically all the oat fields of the State and was responsible for the abnormal condition prevalent in the early part of that season.

DESCRIPTION OF HALO-BLIGHT LESIONS

The halo-blight is most conspicuous on the leaves (Pl. C; 26), although it may occur on leaf sheaths and glumes (Pl. 29). Typical well-developed lesions of the disease are oval chlorotic spots $\frac{1}{2}$ to 2 cm. or more in diameter about points of infection which consist of gray-brown collapsed tissue measuring from 1 to several millimeters in length. The halolike border is at first only slightly lighter green than the surrounding tissue, but as it becomes older it loses more of its green color and forms oval yellow halos about the central infection areas.

Lesions are first visible as light green oval spots 4 to 5 mm. in diameter with central sunken points of infection at first evident only on one side of the leaf. This center of infection increases slowly in size, penetrates

¹ The greater part of this work was carried on at the University of Wisconsin during 1917 and 1918 under the direction of Prof. L. R. Jones and was continued in the Pathological Laboratory of the United States Department of Agriculture during 1918 and 1919 under the direction of Dr. Erwin F. Smith. The writer also wishes to acknowledge the courtesy of the Boston Branch of the Association of Collegiate Alumnae whose research fellowship she held during the college year of 1917-18.

the leaf tissue, and in a day or two forms a gray or brown dry tissue from 1 to several millimeters in diameter, evident on both sides of the leaf blade. The halolike margin spreads rapidly, becoming uniformly lighter green to yellow or showing concentric markings (Pl. 26) of different shades of green and yellow. Occasionally these halolike margins are prolonged at one end into points (Pl. C) from 1 to several centimeters long. They may extend as yellow streaks through the center or along the margin to the tip of the leaf, but ordinarily they appear as oval spots, measuring 1 cm. or more in diameter. Marginal infections are common, forming crescent-shaped lesions. These halolike lesions are conspicuous and characteristic. Except in the central infection area the tissues remain turgid and have a normal appearance except for the paler yellowish color. There is no water-soaked margin about the halo as described by Wolf and Foster (10)¹ for similar lesions of the wildfire disease of tobacco, and the spots do not fall out of the leaves. Exudate does not occur in connection with the lesions. When several lesions occur on the same leaf they often coalesce and produce a general yellowing followed by a breaking across of leaf blades (Pl. C) or a shriveling and drying of tips and margins. During periods of warm, dry weather yellow haloed leaf tissue loses its turgidity and color and forms oval, gray-brown dead spots which on some leaves have narrow, brown margins and on others narrow, yellow halolike margins. Very rarely the dead tissue may assume a pinkish or reddish brown color. In separate lesions the oval outline of the dead halo persists, and even when the whole leaf becomes dry and brown, the original halo outlines may still be distinguished.

PREVALENCE AND GEOGRAPHICAL DISTRIBUTION

Personal observations in Wisconsin and specimens of diseased plants from Ohio, Illinois, Indiana, Minnesota, Tennessee, California, and Virginia have led to the conclusion that halo-blight is present in oat fields every season, scattered lesions occurring on the lower leaves more or less throughout the season and occasionally attacking the panicles. These lesions on the lower leaves are more or less hidden by the fresher upper leaves and so escape observation. Only under particularly favorable weather conditions does the blight develop sufficiently to attract attention or to do serious damage.

FIELD WORK IN 1918

During the season of 1918 weather conditions favorable to halo-blight prevailed in Wisconsin and parts of adjoining States, causing an unusually severe bacterial blighting. In the experimental plots, halo lesions began to appear on from 1 to 25 per cent of the young plants about the middle of May. By May 25 practically every plant showed

¹ Reference is made by number (*italic*) to "Literature cited," p. 172.

some spotting. During the last week in May and the first week in June all untreated plots looked yellowed or slightly browned when viewed from a distance. Practically every first leaf and half of the second leaves were yellowed and dead. Many leaves had yellowed, shriveled tips and margins, and single lesions were abundant on the upper leaves of many varieties.

Every field about Madison showed some blighting. Usually the brown, dead leaves were easily seen from the road, and 100 per cent of infection was not at all uncommon. Some fields south of Madison showed distinct yellow spots from a yard to a rod or more in diameter.

One field of oats near Monroe, Wis., visited May 29, was so badly blighted as to show from a distance a general yellowing with scattered patches of more marked yellow. Closer examination showed abundant halo lesions, every plant being infected. About 3 per cent of the plants were yellowed throughout, the outer leaves were water-soaked and dead, and some whole plants were stunted to such an extent that their recovery seemed doubtful. On the remaining 97 per cent of the plants the outer two to three leaves were collapsed and dead, and the others showed scattered halo lesions in varying stages of development. Where the blight was farther advanced the leaves were broken over and the tips shriveled and brown. Other leaves showed typical, conspicuous, isolated halo lesions which were central or marginal, covering one-half to the entire width of the leaf blade. The plants in this field showed no marked reddening. They had been badly beaten by recent driving storms. Two other oat fields in the vicinity showed a normal stand, but the halo-blight was abundant. No plants remained uninfected, but nevertheless none were stunted or entirely yellowed, and chances for recovery were much better than for the field described above. The blight was general throughout the section about Monroe, and the two fields last mentioned probably represented the average. This yellowed condition of oat fields in this section was first evident May 26 and was reported by a number of farmers.

From May 31 to June 2 oat fields were visited by the writer in five counties of southern Wisconsin. More than 130 fields were inspected, and every one showed halo-blight varying in amount from a fraction of 1 per cent to 100 per cent, the latter being much the more common. The amount varied not only in individual fields but also conspicuously in different counties.

In Jefferson County 26 fields were visited. The oats were about half grown. One-fourth of the fields showed only scattered lesions on the lower leaves—an infection of 1 per cent or less. About one-half of the fields showed a general spotting of the lower leaves on from 60 to 100 per cent of the plants. In some cases the infection was in patches from 2 to 6 feet in diameter, where every plant had all but the last one or two leaves badly spotted. A few fields showed general and heavy infection of 100 per cent of the plants. Even the upper leaves were spotted.

The lower leaves were mostly gone, but a general yellowing of the fields was not marked. Only one field was so seriously affected as to show heavy general blighting and large yellow spots 1 to 3 rods across. About 60 per cent infection of the lower leaves was typical for the fields throughout this section.

In Dodge County the halo-blight was much more abundant. Of the 37 fields visited all showed at least 20 per cent infection; 5 showed light infection—spotting of the lower leaves of 20 to 50 per cent of the plants. This infection, however, was evident from the road. Over half of these fields showed heavy infection—60 to 100 per cent—on at least the lower leaves, and yellowed spots in the fields. The plants in these yellowed spots had little normal green leaf area, and as many as 10 per cent of the plants were entirely yellow and stunted. About one-third of the fields showed 100 per cent infection of the lower leaves, the browned tips and margins showing plainly and often giving a brownish tinge to the fields. In two fields the lower two to three leaves were practically dead and the upper leaves so badly spotted as to give a general yellow color to the fields. In all fields visited in Dodge County blight was evident without a close examination and was sufficiently severe to threaten the crop if unfavorable weather conditions continued. New green leaves were just beginning to appear.

In Fond du Lac County, farther north, the plants were smaller—6 to 8 inches high—and the blight was not heavy in most fields. Seven fields showed only traces of blight on lower leaves—1 to 30 per cent. One showed 100 per cent infection on the lower leaves and another heavy infection—100 per cent—and a general yellowing of the field.

In Columbia and Sauk Counties 10 fields showed a normal blue-green color but had 20 to 100 per cent infection on the lower leaves. Ten other fields showed yellow spots or a general yellowing of the fields. This section was second to Dodge County in the amount of bacterial blight.

Reports and specimens of plants from 35 counties in Wisconsin showed that leaf lesions were general throughout the oat-growing sections of the State and that a single disease, the halo-blight, was responsible for the trouble. A similar condition was reported for the oat fields of southern Minnesota, Iowa, northern Illinois, and Indiana.

For several years previous to 1918 this bacterial blight was observed in Wisconsin oat fields, but there was never enough of it to attract particular attention. The cool, cloudy days and frequent rains of the 1918 oat season proved to be just the conditions necessary to favor the development and spread of the disease. The average rainfall for May, 1918, was 6.66 inches, or considerably more than the normal for that month and greater than for any year since 1892. At Madison there were only four clear days during the month, and at least four heavy rainstorms were accompanied by strong, driving winds especially favorable to the spread of the disease. During June the weather conditions were much

less favorable for the spread of the bacterial blight. The total precipitation in Wisconsin for June was 2.31 inches, or below normal, while the average temperature increased from 58.1° F. in May to 63.9° in June.

With this rise in temperature and decrease in rainfall reports came in of improved conditions in the oat fields. The new leaves which came out were unspotted, and by the last of the month all the fields had resumed a normal color and appeared to have almost completely recovered. The badly yellowed field near Monroe was visited again July 2. It had resumed a normal green color throughout with no halo lesions on the upper leaves and only scattered old lesions lower down. The stand was thin and the plants smaller than in adjoining fields. Neighboring fields were just heading out, but this field would be 10 days to 2 weeks late. Other fields showing yellow spots were reported to have resumed a normal color, but plants in spots previously yellowed were at least a week behind the others in development. This change of weather conditions in June came at an opportune time. Continued cloudy, rainy weather would undoubtedly have destroyed many plants and reduced the yield. As it was, reports for the two seasons of 1917 and 1918 show an increase per acre for the whole State of 2.2 bushels in 1918, but this increase would undoubtedly have been more than doubled but for the presence of halo-blight. Following the unusually severe bacterial blight of the early part of the season, blasting of panicles was also unusually abundant and general throughout Wisconsin oat fields during 1918. In extreme cases as many as 25 to 50 per cent of the spikelets in a head were undeveloped. Counts of 30 panicles in a severely blighted spot gave an average of 29 spikelets per panicle and 31 per cent blasting. Counts of 30 panicles from a part of this same plot not severely halo-blighted gave an average of 34 spikelets per panicle and 20 per cent blasting.

On six panicles sent in from Lincoln County the numbers of normal and blasted spikelets were as follows:

Panicle No.	Number of normal spikelets.	Number of blasted spikelets.
1	36	28
2	24	^a 34
3	38	28
4	10	8
5	34	20
6	16	19

^a Top blasted.

The blasted spikelets are mostly in the lower half of the panicle, but occasionally the upper half is blasted as in No. 2.

All the experimental plots showed considerable blasting and numerous empty spikelets. Counts of 36 panicles of Wisconsin No. 14 oats from treated seed showed an average of 11 per cent of the spikelets blasted,

varying from 0 to 30 per cent. Counts of 40 similar panicles showed 21 per cent of the spikelets blasted. Experiments carried on during the summer of 1918 indicate that this blasting is probably not due to the bacterial disease but to the unusual meteorological conditions which favored the development and spread of the bacterial blight.

BACTERIAL ISOLATION EXPERIMENTS

Oat plants showing typical lesions of halo-blight were collected from fields around Madison, Wis., and from other points in the State, from Tennessee, Urbana, Ill., Lafayette, Ind., Wooster, Ohio, Davis, Calif., and Arlington Farm, Va. Twenty-eight isolations were made from these lesions, and 36 isolations from halo lesions produced by inoculations in the field and greenhouse. Most of these isolations were from leaf lesions, but a few were made from lesions on glumes (Pl. 29).

The first isolations were made by washing the leaf tissue through 10 sterile water blanks, crushing on a sterile slide, transferring to broth, and plating from this broth suspension. Later isolations were made by dipping the tissue for a second in 95 per cent alcohol, then into 1 to 1,000 mercuric chlorid (HgCl_2) for one minute, washing through three sterile water blanks, and proceeding as in the earlier method. This later method proved to be more satisfactory, but a comparison of the results from both methods proved interesting.

From all these isolations, with the exception of two from glumes, typical white colonies of the halo organism were obtained. These appeared on potato agar in from 1 to 3 days. When the first method of isolation was used, without sterilizing the surfaces of the tissues, yellow colonies appeared on the plates with the white colonies in 25 per cent of the isolations from natural infections and in 22 per cent of the isolations from inoculation experiments. When the surfaces of the lesions were sterilized in mercuric chlorid for one minute no yellow colonies were obtained. Twelve isolations were made from natural infections, using mercuric chlorid; and a still larger number were made from lesions due to inoculation experiments. One set of isolations was made by placing the leaf tissue in the mercuric chlorid for only 30 seconds. This leaf tissue had been sprayed with a mixed culture of yellow and white organisms. Yellow colonies appeared on the plates with the white colonies, but the yellow colonies were not nearly so numerous as on plates poured from tissue which had not been sterilized. If the tissue had remained in the mercuric chlorid for 60 seconds instead of 30 seconds no yellow colonies would have appeared. These yellow organisms appear to be surface saprophytes and do not occur within the tissues.

The yellow colonies were mostly of one kind, judged by their appearance on agar plates—round, smooth, shining, lemon-yellow with entire margins—and they appeared on the potato agar in from one to two days. This type of colony was chosen for inoculation experiments.

The white colonies of halo-producing organisms from natural infections were all alike on beef-peptone agar, but on potato agar two only of the many isolations gave colonies of a slightly different character, like that designated in this paper as "stock."

On potato agar most of the isolations gave raised, umbonate colonies of a butyrous consistency with thin margins, entire or slightly undulate. This was the usual type of colony isolated. The two varying isolations were from a leaf lesion from Lafayette, Ind., and a glume lesion on Wisconsin No. 14 oats in an experimental plot. (See Pl. 31, C; 32, A.) The colonies were thicker and of an equal thickness out to the margin; the margin was slightly undulate, and the consistency of the colony was like that of boiled starch or gelatin. They gave a more rapid and abundant growth on potato agar than the common type. This second type of colony is the same as an isolation made in 1916 by Mr. Reddy from a halo lesion on oats and kept as a stock culture at Madison, Wis.

The pathogenicity of each of these 28 isolations from natural infections was tested and proved by one or more inoculation experiments. Mr. Reddy's oat stock culture and isolation No. 36 (the common form) from a leaf lesion from Wooster, Ohio, were used as representatives of the two types of white colonies in the inoculation and cultural work and are designated respectively as "stock" and "36."

INOCULATION EXPERIMENTS

1. Inoculation experiments were carried on at Madison, Wis., in experimental plots out of doors and in the greenhouses. The plants in the field were in various stages of development, from half grown to fully headed; and those in the greenhouse were from 4 to 8 inches high. The uninjured plants were sprayed with water suspensions of organisms from agar slants 2 days to 1 week old. The greenhouse plants were then placed in damp chambers for 48 hours. Plants sprayed in the field were covered with water-proofed translucent (glassine) bags for the same length of time. Control plants were sprayed with sterile water and treated in the same manner. Oat plants of Wisconsin No. 1, Wisconsin No. 5, and Wisconsin No. 14 were used for greenhouse inoculations. Wisconsin No. 14 was used more often than the others because it proved to be more susceptible than any other variety. Occasionally halo lesions appeared at the end of the first 48 hours, when the plants were removed from the damp chamber; but usually none appeared until 3 to 4 days after inoculation. On young plants the lesions were often so numerous that centers of infection appeared in rows where the organisms had entered the stomata. The halolike discolorations around these points of sunken tissue were at first only slightly lighter green than the normal tissue but quickly became more marked until about a week after inoculation, when the tissue was a distinct yellowish green

to yellow. Numerous confluent lesions quickly killed the leaf tips and margins, which shriveled, turned brown, and died. Isolated lesions developed in the same way into distinct oval spots of yellow tissue 1 cm. or more in diameter with small dead centers. Infection was always abundant on inoculated oat plants. (See Pl. 27.)

Cultures proved by inoculation experiments to be pathogenic were kept as stock cultures. In this way 21 such cultures were obtained.

2. Since both yellow and white colonies were isolated from leaf sections showing halo lesions, inoculations were made with pure cultures of each and also with mixed cultures of yellow and white colonies for comparison with inoculation work done by Thomas F. Manns (3, *p.* 107, *Pl. I*). In 25 inoculation experiments pure cultures of the white halo organisms produced abundant and typical infections. In 13 tests, pure cultures of the yellow organisms produced no lesions whatsoever. Twelve sets of inoculations were made with mixed cultures by combining the 2 white halo organisms, No. 36 and stock, with 4 different isolations of yellow organisms. Isolation 39a from a leaf lesion from Urbana, Ill., was the yellow organism most often used. Separate pure cultures of yellow and white organisms were used for control inoculations. The cultures were mixed just before the inoculations were made for the reason that long-continued attempts to grow mixed cultures in broth or on various agars were not successful.¹ In the 12 inoculation tests with the yellow and white mixed cultures typical halo infections were produced, but the lesions were only one-half to three-fourths as abundant as on plants inoculated with pure cultures of the white organisms. The development of lesions from mixed cultures was also somewhat retarded, the infections being evident from one to two days later than those obtained from the pure white cultures. These inoculation experiments showed plainly that the white organism alone is responsible for the production of the halo lesions while the yellow organisms used are neither parasites nor favorable to parasitism.

3. In June, 1918, field inoculations were made on the following 13 Wisconsin varieties: Wisconsin No. 1, 3, 4, 5, 7, 13, 14, 15, 22, 25, 49, 52, and 62. The plants were just beginning to head out, and the experiment was carried on to test the pathogenicity of the white organisms on mature leaves and on panicles, and the effects of possible lesions upon the development of the panicles, spikelets, and kernels. Water suspensions of the halo organisms were sprayed into unopened sheaths upon uninjured bundles of plants, the tops of which were drawn together and tied close so as to be covered with bags, and upon bundles of plants

¹ For two months mixed cultures of white and yellow organisms were grown on potato agar and in +10 beef-peptone broth. Plates poured from these cultures when they were 5 days old showed a few white and many yellow organisms in the broth cultures and about equal numbers of yellow and white on agar. Plates poured from these cultures 7½ weeks later showed no growth of either white or yellow colonies from the agar and showed pure cultures of the yellow organisms from the broth. On the contrary, separate pure cultures of the same organisms held for the same time in these media and under the same conditions gave abundant and characteristic colonies on the plates poured.

injured with a scalpel or drawn between the fingers to rub off the bloom. Bundles of control plants were treated in the same manner and sprayed with sterile water. All inoculated and control plants were covered with glassine bags for 48 hours, as stated above. Characteristic halo lesions appeared on all the varieties inoculated except Wisconsin No. 4. Only uninjured plants of this variety were inoculated. Five other varieties (No. 22, 25, 49, 52, and 62) showed no lesions on uninjured plants, but all varieties showed fairly abundant spotting of leaves and sheaths of plants which had had the bloom removed or had been cut with a scalpel. Some of these leaves were almost entirely yellowed with lesions. On 6 varieties lesions appeared on uninjured plants, but the lesions were not nearly so abundant as on injured leaves and panicles. Wisconsin No. 7 was the only variety in which the panicles were entirely out of the sheaths. In this variety every spikelet of the injured panicles showed halo lesions which stood out as oval yellow spots on the glumes. About half of the spikelets in these panicles were not filled out. Spikelets of untreated panicles of the same variety were also poorly filled out. Under favorable conditions the panicles appear to be just as susceptible to halo-blight as the leaves. Wisconsin No. 14 also showed heavy spotting of injured panicles. Uninjured spikelets of two varieties were halo-spotted when the suspension was sprayed into the unopened sheath.

Though none of the controls showed any halo lesions, both water-sprayed controls and inoculated plants showed considerable sterility, amounting to from one-fifth to one-half of the spikelets in a panicle. Untreated heads of the same varieties and in the same plots showed either no sterility at all or only traces at the base of the panicle. This sterility was particularly abundant when either the water suspension or sterile water was sprayed into unopened sheaths or sheaths just opening at the top. The fact that both controls and inoculated plants showed the same amounts of sterility would indicate that the sterility was not due to the effects of the organism. Excessive moisture around the developing spikelets while these were still inclosed within the sheath offers the most plausible explanation for this sterility. In the same way heavy rains at the time oat fields are heading out probably account for the sterility commonly observed in oat fields. This set of field inoculations has led to the following conclusions:

1. Leaves and panicles of oat plants approaching maturity are susceptible to halo infection under favorable conditions.
2. Infection takes place more readily on injured than on uninjured parts of the plants.
3. Some varieties are more susceptible to infection than others. Greenhouse inoculations on young plants also led to this conclusion.
4. Although both natural and artificial halo infection may occur on heads, these infections are not responsible for the blasting of oat heads. Sterility is due probably to physiological rather than pathological conditions.

CULTURAL CHARACTERS

I.—STOCK HALO ORGANISM

MORPHOLOGY.—The organism is a motile rod with rounded ends (Pl. 34, B, E), sometimes occurring singly or paired, but usually in short to long chains (Pl. 34, C). Organisms grown on beef-peptone agar and potato agar and stained with Ribbert's capsule stain, gentian violet, and carbol fuchsin measure from 1 to 4 μ in length and from 0.4 to 0.8 μ in width, with an average of 0.65 by 2.3 μ . Stained by the Van Ermengen method from 24-hour cultures on beef-peptone agar, the organism shows from one to several polar flagella about the same length as the organism or only a little longer (Pl. 34, E). No spores have been observed, although special staining methods with hot carbol fuchsin and methylene blue were used. Capsules are formed on both potato and beef-peptone agar and were stained with Ribbert's capsule stain (Pl. 34, D). Compact pseudozoogloae are not formed—that is, there is little or no viscosity. No branched forms have been observed.

NUTRIENT BROTH.—Beef-peptone bouillon (+10) shows light clouding in 24 hours at 25° C. In 5 days there is moderate uniform clouding, and a flocculent white film or pellicle forms on the surface and falls to the bottom of the tube in small white flakes. On further shaking the flakes disappear. In older cultures there may be no pellicle but merely a slight ring around the surface. The clouding is never very heavy, and the thin surface film soon disappears. The medium is gradually changed in color until at the end of 60 days it is a deep amber brown.¹ The odor of decay is distinct with more or less of the penetrating smell of ammonia. The sediment in cultures from recent isolations is loosely flocculent. There was a somewhat viscid swirl in some of the old broths containing sodium chlorid. Rectangular crystals form at the surface.

BROTH PLUS ABSOLUTE ALCOHOL.—To 10 cc. of +15 beef-peptone bouillon absolute alcohol was added to make 4, 5, 6, and 7 per cent. There was heavy clouding in 4 and 5 per cent, moderate clouding in 6 per cent, and slight clouding in two out of three tubes of 7 per cent.

AGAR STROKE.—On +10 beef-peptone agar slants growth in 2 days is moderate, flat, undulate, white, shining, translucent, slightly contoured, butyrous. The medium is slightly browned. There is a slight odor of decay.

On potato-dextrose agar slants the growth in 2 days is abundant, slightly undulate, raised, glistening, smooth, opaque, white, of gelatinous consistency (Pl. 30, B, b). The medium is unchanged and there is no odor.

AGAR COLONIES.—(1) On poured plates of +10² beef-peptone agar from +10 broth cultures, colonies appear after 30 hours at 25° C. as tiny translucent dots. When 2 days old the colonies are 1 to 2 mm. in diame-

¹ RIDGWAY, ROBERT. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D.C. 1912.

² Fuller's scale.

ter, white, smooth, shining, round, with a denser center. When 11 days old they are more or less irregularly circular, 5 to 10 mm. in diameter in thin-sown plates, and flat with slightly raised margins. Microscopically, with low powers, the internal structure is filamentous, the margin consisting of folded parallel strands or chains. The margin is undulate. Deep colonies are lens-shaped and opaque. The medium may be slightly browned. The markings in Plate 31, A, B, E, F, are characteristic of these colonies under a hand lens, and they do not disappear as the colonies grow older. Very similar markings appear in young colonies of some of the softrot organisms. A half dozen of these softrot organisms tried on oats have not produced any halo lesions.

(2) On plates of potato-dextrose agar the colonies grow more rapidly. They are raised, white, shining, opaque, with only slightly undulate or entire margins, and of the same gelatinous consistency described above (Pl. 31, C; 32, A.)

GELATIN COLONIES.—Growth slow, circular, crateriform, margins entire to undulate with folded strands (Pl. 31, G), liquefaction saucer-shaped, rather slow.

GELATIN STAB.—Growth on +10 peptone gelatin at 22° C. is slow, best at the top, with only a slight filiform growth along line of stab, liquefaction crateriform. At the end of 2 days a small pit is formed at the surface 3 mm. deep. At the end of 6 days liquefaction extends two-thirds of the way across a 20-mm. tube, and the pit is 10 mm. deep. At the end of 18 days liquefaction covers the surface to a depth of 14 mm. At the end of 60 days (at 20°) the tube is only half liquefied.

POTATO CYLINDERS.—At 25° C. there is moderate growth in 24 hours and slight darkening of the medium. At the end of 4 days growth is abundant, flat, smooth, glistening, butyrous to slimy, of a cream color, and the medium is a uniform dark gray color. At the end of 20 days there is a decided odor of decay. There is feeble diastasic action on starch.

SMITH'S POTATO STARCH JELLY (5).—Growth moderate, diastasic action feeble, medium stained a light bluish green. Traces of dextrin.

STARCH AGAR.—To melted tubes of +10 beef-peptone agar sterile potato starch was added and plates poured. Tests with iodine showed no diastasic action.

LITMUS SUGAR AGARS.—One per cent lactose, maltose, dextrose, saccharose, and galactose were used in beef-peptone litmus agar. Change of medium to a bright red showed considerable acid production with dextrose and galactose; with saccharose there was less acid produced; and with lactose and maltose there was no evidence of acid production. Reduction of litmus took place to a slight extent under the streaks with dextrose and maltose.

MILK.—In fresh isolations a soft curd forms in from 5 to 7 days, followed by slow peptonization of the curd, which is completed in from 5

to 6 weeks. In old isolations curd usually is absent. The medium does not become viscid or slimy. The liquid at the top of the tube is sometimes a yellowish green but more often brown. This brown color may be confined to a surface layer a few millimeters deep or may extend throughout the liquid medium. In old tubes the fluid is coffee-colored. It is unlike any color in Ridgway, but is somewhat like his moss brown.

LITMUS MILK.—At room temperature the medium begins to turn slightly blue in 2 days, beginning at the top; and in 5 or 6 days it is frequently stratiform, being deepest blue at the top. Reduction begins at the end of a week, the tubes becoming cream-colored throughout, and clearing at the top or showing reduction only at the bottom of the tube for a depth of 1 cm. or more. There is no curdling, and clearing is complete in 2 weeks. At the end of 2 months the tubes are a deep blue-black and sometimes of a gelatinous consistency. At no time is there any red-dening.

METHYLENE BLUE IN MILK.—In fresh isolations reduction begins in 3 days and is completed in 7 days, except for a rim of blue at the top 1 mm. deep. Curdling takes place in 1 week; peptonization begins soon after and is completed in 5 weeks, the clear liquid being yellowish to neuvidier green, especially toward the top.

COHN'S SOLUTION.—Growth is very slight, appearing in 24 hours and increasing slightly the second day. In a week clearing begins, and at the end of 3 or 4 weeks there is no clouding and only a little precipitate. Nonfluorescent. No crystals.

USCHINSKY'S SOLUTION.—The medium shows light clouding in 24 hours. In 48 hours a thin flocculent white film has formed over the surface and shakes down in fine particles. In 4 days there is moderate clouding, a slight surface film, and the medium is a pale turtle green. In 2 weeks a heavy white rim has formed around the surface of the liquid. When the cultures are 6 weeks old there is considerable white precipitate—fluid, not viscid—and slides stained in carbol fuchsin show a network of long chains (Pl. 34, C). Fluorescence persists in old cultures.

FERMI'S SOLUTION.—Light clouding occurs in 24 hours. In 4 days there is moderate clouding and a delicate surface film which shakes down in fine flocculent particles. In 4 days as much growth as in Uschinsky. In 2 weeks the clouding is heavy, the medium has a greenish tinge, and there is a heavy white surface pellicle 2 to 3 mm. deep, which shakes down in strings of fine white particles. There is considerable white precipitate—a heavy growth. In 3 weeks the white surface pellicle and the precipitate become cream-colored. No chains are formed. Greening first visible after about 2 weeks. At end of a month surface pellicle and precipitate tan color. Clouding and pellicle twice as abundant as in Uschinsky.

LOEFFLER'S BLOOD SERUM.—Growth moderate, filiform to slightly undulate, flat, glistening, smooth, medium slightly browned beneath the streak. No liquefaction, not even after 2 months.

SOYKA'S RICE MEDIUM.—The growth and medium in all cases except one were cream colored. A culture marked "stock b" turned the medium a buff-pink.

NUTRIENT BROTH PLUS CARBON COMPOUNDS.—To tubes of +10 beef-peptone bouillon 1 per cent asparagin was added and to other tubes 1 per cent asparagin plus 1 per cent dextrose were added. The growth was equally good in both kinds of media. The organisms seem to obtain their carbon as readily from asparagin as from dextrose.

INDOL PRODUCTION.—Feeble or absent in beef-peptone bouillon or 1 per cent peptone water containing 0.5 disodium phosphate and 0.1 magnesium sulphate.

HYDROGEN SULPHID.—Hydrogen sulphid is not produced. Lead acetate paper suspended over broth cultures is not blackened, and the medium is unchanged when streaks are made on lead carbonate agar plates.

AMMONIA PRODUCTION.—Moderate. Made tests with Nessler's reagent.

NITRATE IN NITRATE BROTH.—No gas is produced in fermentation tubes. Nitrates are not reduced. Tests were made at the end of 9 days and at the end of 2 months.

TEMPERATURE RELATIONS.—The maximum temperature for growth, tested on beef broth and on agar and potato, is 31° C. The minimum temperature for growth is below 0°. Tubes surrounded with ice showed clouding. The optimum temperature for growth is 24° to 25°. The thermal death point is between 47° and 48°.

MOISTURE RELATIONS.—The organisms are very readily killed by drying. Smears were made from 5-day-old broth cultures to sterile cover glasses and placed in sterile Petri dishes. Pieces of these cover glasses transferred to sterile bouillon after 3 hours showed growth in all. All were dead at end of 24 hours. In a repetition, transfers after 6 hours gave no growth.

FERMENTATION TESTS: (1) POTATO JUICE.—Undiluted potato juice was expressed after passing the pared tubers through a meat grinder. Moderate clouding in open arm of fermentation tubes. No growth in closed arm and no gas.

(2) MILK.—At the end of a week the milk at the open end had cleared without evident curdling. Two days later the milk in the closed end had curdled. This curd was gradually peptonized, about a third of it remaining at the end of 2 months. The cleared liquid in the open arm was browned—a chestnut to auburn brown at the surface and gradually changing to a lighter shade through the open arm and a third of the way up the closed arm. No gas was formed.

(3) CARBON COMPOUNDS.—Tests were made in the fermentation tubes with 2 per cent solutions of dextrose, saccharose, maltose, lactose, mannit, glycerin, and levulose in 2 per cent water solutions of Difco's and Witte's peptones. *Bacillus coli* Escherich was used as a control and produced gas in the closed arm. The oat organism produced no gas and

did not grow in the closed arms of tubes containing maltose or lactose. In tubes containing saccharose, glycerin, and mannit there was growth at first only in the open arm, with a sharp line of demarcation between open and closed arms. At the end of a week clouding began to appear in the closed arms of tubes containing these three substances. In 3 weeks there was light clouding throughout the closed arms in saccharose, moderate clouding throughout the closed arms in mannit, and in glycerin heavy clouding to within an inch of the top of the closed arm with light clouding on up to the top. In a later test there was again light clouding throughout the closed arms of tubes containing saccharose. In two later tests a moderate clouding appeared in the closed arms of tubes of saccharose and dextrose in from 4 to 7 days. In a later test of mannit and glycerin there was no clouding in the closed arm. Tests for ammonia with Nessler's reagent gave a positive reaction in solutions of maltose, saccharose, mannit, glycerin, and lactose, but only traces of ammonia or negative reactions in cultures containing dextrose. Titrations with phenolphthalein as an indicator show a higher total titrable acidity in the cultures than in the controls in saccharose and dextrose. These solutions were also acid to litmus as compared with controls in two later experiments. The hydrogen-ion concentrations, determined after about 6 weeks by the colorimetric method, were as follows: Control, $P_H=4.8$; dextrose, $P_H=4.8$; maltose, $P_H=7$; saccharose, $P_H=6.4$; and lactose, $P_H=4.8$.

The organisms grew best in saccharose, levulose, and dextrose, showing heavy growth in the open arm and slight to moderate growth in the closed arm. This organism is evidently a facultative anaerobe when certain sugars are available.

TOLERATION OF ACIDS.—Transfers were made to tubes of +10 beef-peptone broth containing 0.1 per cent and 0.2 per cent of citric, tartaric, and malic acids. There was good growth in 0.1 per cent of each acid but only slight growth or none at all in 0.2 per cent.

TOLERATION OF SODIUM CHLORID.—Neutral beef-peptone bouillon containing, respectively, 2, 3, 4, 5, 6, and 7 per cent of sodium chlorid was inoculated from potato agar slants. There was slight clouding of 2 per cent after 3 days. None of the stronger solutions clouded, but slides made from a stringy white precipitate and stained with carbol fuchsin showed that long chains of cells had been formed in all strengths of sodium chlorid. A second test was made, using neutral broth with 0.5, 1, 1.5, 2, 3, and 4 per cent solutions of sodium chlorid and inoculating from broth cultures. There was slight clouding in 1 per cent at the end of 2 days, slight clouding in 1.5 per cent at the end of 3 days, moderate clouding in 1.5 per cent at the end of 5 days. At the end of 7 days there was slight clouding in 2 per cent and moderate clouding and a stringy swirl of precipitate in 2 per cent at the end of 19 days. Stained slides of precipitate from 1.5 and 2 per cent solutions showed a network of long chains. In the second test there was no growth in solutions of more than 2 per cent.

OPTIMUM REACTION AND TOLERATION LIMITS.—Beef-peptone bouillon was adjusted to each of the following reactions with sodium hydroxid and hydrochloric acid +20, +15, +10, +5, 0, -5, -6, -13, -16, and -22. These were uniformly inoculated from broth cultures and kept at 24° C. At the end of 24 hours there was light clouding in -5, 0, +5, and +10. Subsequent clouding occurred in -6 and +15. A stringy precipitate formed in -13, and on -15 a thin surface film developed and the medium was slightly darkened. At the end of 48 hours the clouding in +5 was slightly heavier than in +10, and the flocculent surface film slightly heavier. Clearing began in 3 weeks. At that time +10 was browned, +15 slightly deeper brown, and +5 and -5 showed a greenish tinge. The optimum reaction for growth is, therefore, +5 Fuller's scale, although +10 and +15 are also favorable reactions.

In later tests the limits of growth on agar were +27 and -17, and in bouillon +27 and -18, when the agar was reinoculated from an alkaline culture.

VOLATILE ACIDS.—Tests for volatile acids were negative. Cultures were grown in tap water containing 1 per cent Witte's peptone and 1 per cent dextrose. The steam from these cultures gave an alkaline reaction to litmus although the liquid was acid to litmus.

FREEZING.—Six plates were poured in +15 agar from 24-hour +15 broth cultures. This 24-hour culture was exposed for 1 hour in salt and crushed ice and then six more plates were poured. Eighty-seven per cent of the organisms were killed by this treatment.

EFFECT OF SUNLIGHT.—The organism is sensitive to sunlight; 80 per cent were killed by 15 minutes' exposure on ice in thinly sown beef-peptone agar plates.

VITALITY ON CULTURE MEDIA.—Typical colonies of this organism have been obtained from +10 beef-peptone agar slants which have stood for 11 months and from broth cultures 10 months old. These were tested by inoculation on young oat plants and gave abundant and typical halo lesions.

LOSS OF VIRULENCE.—Loss of virulence on culture media has not been observed in cultures carried for more than 3 years.

GROUP NUMBER.¹—221.2323023.

The name *Bacterium coronafaciens*, n. sp., is suggested for this organism.

TECHNICAL DESCRIPTION

Bacterium coronafaciens, n. sp.

A motile rod with rounded ends and polar flagella; single, in pairs or long chains, average measurement 2.3 by 0.65 μ ; no spores, zoogloea, or involution forms; capsules are formed; slightly facultative anaerobic. On nutrient agar colonies are white, round becoming irregularly circular, flat with slightly raised margins, surface smooth or slightly contoured; deep colonies are lens-shaped and opaque. Its proteolytic

¹ SOCIETY OF AMERICAN BACTERIOLOGISTS. DESCRIPTIVE CHART. Indorsed by the society for general use at the annual meeting Dec. 31, 1914. Prepared by the committee on revision of chart identification of bacterial species.

power is moderate; gelatin is liquefied slowly, beginning in 2 days and not complete in 60 days; reduction of litmus occurs in milk, and the casein is digested without curdling; milk curdles in 5 days, and peptonization is completed in 5 weeks. No acid is produced in milk. Oxidations of proteins are incomplete; ammonia is produced; hydrogen sulphid, gas, and indol are not produced. Nitrates are not reduced. There is slight diastasic action on potato cylinders. Good growth in Uschinsky's solution and in Fermi's solution. Growth in Cohn's solution is scanty. Maximum temperature for growth is 31°C ., minimum below 0° , optimum 24° to 25° , thermal death point between 47° and 48° . Tolerates sodium hydroxid to -18 Fuller's scale and hydrochloric acid to $+27$. The optimum reaction for growth is $+5$ Fuller's scale. Gram-negative, not acid-fast, stains readily and uniformly with gentian violet and methylene blue. Stains more or less irregularly with carbol fuchsin (often polar staining). Sensitive to drying; 87 per cent killed by freezing, 80 per cent killed by sunlight. Vitality on culture media long. Pathogenic on varieties of cultivated oats and to a slight degree on wheat, rye, and barley, producing oval halolike lesions of chlorotic tissue surrounding dead brown centers of infection.

Beef-peptone agar and beef bouillon are favorable media for prolonged growth. Growth on potato agar brings out more distinguishing characteristics.

II.—ISOLATION NO. 36

This isolation was made from a halo lesion on oats obtained from Wooster, Ohio, in June, 1917. It has the same group number as the stock halo organism just described but differs from it in the characters mentioned below. The differences, though not very marked, seem to be fairly constant, while the lesions from which the cultures were isolated and which they produce in inoculation work can not be distinguished. The stock organism seems to be slightly more virulent.

MORPHOLOGY.—The organism occurs singly or in twos but seldom in long chains (Pl. 34, A). Stained by Ribbert's capsule stain it measures from 1.1 to $3\ \mu$ in length and from 0.5 to $0.8\ \mu$, in width, not including the capsule, with an average measurement of 0.66 by $2.1\ \mu$.

BEEF AGAR PLATES.—On $+10$ beef-peptone agar, the surface colonies remain round, and the margin tends to remain entire (Pl. 31, D).

POTATO-DEXTROSE AGAR STROKE.—Two-day-old slants from broth show moderate flatter growth, which is filiform and dull, with more or less wrinkling on the surface. The growth is somewhat translucent and of a butyrous to slightly membranous consistency (Pl. 30, B, a).

GELATIN STAB.—Liquefaction is more rapid, being complete in 40 days.

TOLERATION OF SODIUM CHLORID.—Same as stock, but slides from a 2 per cent solution stained with carbol fuchsin show only a few scattered short chains.

LITMUS MILK.—Litmus is not reduced.

METHYLENE BLUE.—Digestion of casein a little slower than with stock.

USCHINSKY'S SOLUTION.—No chains on slide stained with carbol fuchsin.

COHN'S SOLUTION.—Clouding heavier than with stock. Crystals are formed on the sides of the tubes.

STARCH AGAR.—The organism showed a feeble diastasic action on starch.

TEMPERATURE RELATIONS.—Thermal death point is between 47° and 48° C.

Strain 36 usually gives a greenish tinge to bouillon cultures, which in old cultures contrasts strongly with the brown of old "stock" cultures. On ordinary beef-peptone agar the two strains can not be distinguished but on potato-dextrose agar there is considerable difference in amount of growth, and they are noticeably different in consistency. The most important differences perhaps are in size and in nonformation of chains. The rods of No. 36 are shorter and plumper. They seem to be two strains of the same organism.

III.—YELLOW ORGANISM

MORPHOLOGY.—The organism is a motile rod with rounded ends and one to several polar flagella. It occurs singly or in short chains. When grown for 24 hours on beef-peptone agar and stained by the Duckwall modification of Pitfield method, it has an average measurement of 3.5 by 1.4 μ , varying in length from 2.3 to 3.7 μ , and in width from 0.98 to 2.1 μ . No spores have been found.

BEEF-PEPTONE AGAR PLATES.—Colonies appear after 24 hours on +10 beef-peptone agar; in 2 days they measure 2 mm. in diameter and are a translucent light yellow. When a week old, surface colonies are circular, 4 to 5 mm. in diameter, raised, smooth, lemon-yellow, with entire translucent margins. Microscopically the internal structure is finely granular. Deep colonies are lens-shaped and opaque.

BEEF-PEPTONE AGAR STROKE.—Growth in two days is moderate, filiform, flat, glistening, slightly contoured, translucent, light orange-yellow, with a faint odor. Consistency is butyrous, and medium is unchanged (Pl. 30, B, c). The organism lives at least three or four months on beef-peptone agar.

POTATO AGAR STROKE.—In two days the growth is abundant, filiform, flat, spreading, glistening, smooth, opaque, light orange-yellow. The medium is unchanged, and the consistency butyrous.

GELATIN STABS.—At 22° C. growth in +10 nutrient peptone gelatin is moderate. The liquefaction at first is saccate along the stab and later stratiform. Liquefaction is completed in 40 days. The surface growth has a pinkish tinge, but the precipitate is yellow.

BEEF-PEPTONE BROTH.—There is moderate clouding in +10 beef-peptone broth in 24 hours at 25° C., very heavy clouding in 48 hours, and a slight flocculent surface growth. In 3 days there is a heavy membranous pellicle which breaks up when shaken and sinks to the bottom of the tube. The precipitate is abundant and finely granular. Clearing begins in about 2 weeks.

TOLERATION OF SODIUM CHLORID.—Tables of neutral beef-peptone bouillon containing respectively 2, 3, 4, 5, 6, and 7 per cent of sodium chlorid were inoculated from potato agar slants. In 24 hours there was

clouding in 2, 3, 4, and 5 per cent solutions. In 3 days there was a very slight clouding in 6 and 7 per cent solutions. A stringy yellow precipitate formed in the 4, 5, 6, and 7 per cent. Slides made from 2 and 3 per cent solutions and stained with carbol fuchsin showed long chains of cells. There were no long chains in the 4, 5, 6, and 7 per cent. In a second test a delicate pink surface film, not previously observed, formed in 0.5, 1, 1.5, 2, 3, and 4 per cent solutions; and a pink stringy precipitate formed in 2 and 3 per cent, becoming a brick red in 4 per cent.

POTATO CYLINDERS.—At 25° C. there was slight growth in 24 hours and a slight graying of the medium. In 4 days there was abundant yellow growth, and the medium had become slightly browned. Growth was filiform, flat, raised, glistening, somewhat contoured, orange-yellow to red on top. There was no odor, and the consistency was butyrous. There was no action on the starch.

MILK.—Milk titrating +18 on Fuller's scale was inoculated from 9-day-old potato agar slants. A slight yellow surface film was formed in 2 days. At the end of 1 week yellow precipitate was evident. Curdling began in 3 weeks. There was a slight separation of curd and whey at the end of 2 months. The solid curd gradually dried down without any evidence of peptonization.

LITMUS MILK.—Complete reduction occurs in 24 hours, leaving the medium cream-colored. Shaking tended to restore the color. After about a week some of the reduced tubes were steamed, whereupon the original lavender color returned. Curdling occurred in 3 weeks. There was no evidence of digestion at the end of 2 months.

METHYLENE BLUE IN MILK.—Reduction takes place in 24 hours. In 3 weeks there is curdling and the blue color begins to return at the tops of the tubes. No peptonization.

USCHINSKY'S SOLUTION.—There is moderate clouding in 24 hours at 25° C. and a membranous surface film. At the end of 2 days there is a fairly heavy light yellow surface film. In 4 days there is heavy clouding and a heavy surface film and yellow precipitate. Slides stained with carbol fuchsin show many short chains.

FERMI'S SOLUTION.—There is moderate clouding in 24 hours at 25° C. In 2 days there is a fairly heavy light yellow surface film. In 4 days the clouding is heavy and there is a heavy orange-colored surface film 2 mm. thick. At the end of a week this pellicle is 4 mm. thick. Clearing begins in 2 weeks, and yellow strands extend from the heavy pellicle to the bottom of the tube. At the end of 3 weeks the pellicle is 1 cm. thick. In 4 weeks the medium has a greenish tinge. No chains were observed on slides stained with carbol fuchsin.

COHN'S SOLUTION.—There is a slight clouding at the end of 2 days at 25° C. At the end of 4 days the clouding is still very light, and there is just a trace of surface growth. Rhomboid crystals are formed on the tube above the liquid. Growth is very slight in comparison with that

BLOOD SERUM.—Growth was moderate, filiform, slightly raised, orange-yellow, smooth, shining. In 2 weeks the center of the growth became red, but the author was unable to verify this change in 1919. The medium was unchanged.

LITMUS SUGAR AGARS.—In 24 hours there is a slight reddening of litmus dextrose agar and in 3 days reduction has begun in the lower end of the tubes, the upper two-thirds being rose red. Litmus-lactose and litmus-maltose agar show reduction in the lower ends of the tubes in 3 days. These tubes are red through the center and blue at the top. At the end of a week all agars are colorless at the bottom of the tubes, red in the center, and blue toward the top. Growth is abundant. At the end of 2 weeks the colony begins to turn red.

STARCH AGAR.—There is no diastasic action on starch.

INDOL.—Indol production is feeble.

NITRATE BOUILLON.—No gas is produced in fermentation tubes. Nitrates are not reduced.

AMMONIA.—Ammonia production is moderate.

HYDROGEN SULPHID.—No hydrogen sulphid is produced. Tests were made with lead-acetate paper over broth and with lead-carbonate agar.

OPTIMUM REACTION AND TOLERATION LIMITS.—By the use of sodium hydroxid and hydrochloric acid, using phenolphthalein as indicator, beef-peptone bouillon was adjusted to each of the following reactions: +25, +20, +15, +5, 0, -5, -6, -13, -15, and -22. These were uniformly inoculated from broth cultures and kept at 24° C. In 24 hours there was clouding in all except +20 and +25. At the end of 3 days there was clouding in all except +25. The clouding in +20 was slight. At the end of 1 week there was no growth in +25, light clouding in +20, -15, and -22, and heavy clouding in all the other reactions, with precipitation and surface growth. In 3 weeks there was clearing in -15 and -22, but a viscid yellow precipitate. There was never any growth in +25. The optimum reaction for growth is +5 Fuller's scale.

GAS FORMATION AND AEROBISM.—Tests were made in fermentation tubes in the presence of the following carbon compounds: dextrose, saccharose, lactose, maltose, mannit, and glycerin. A 2 per cent solution of each was made in a 2 per cent water solution of Difco peptone. *Bacillus coli* Escherich was used as a control and produced gas in each solution. No gas was produced by the yellow organism. There was clouding in the open arm of all tubes in 2 days, the heaviest growth being in saccharose and maltose. In 3 days clouding began in the closed arm of tubes containing saccharose and mannit. At the end of a week there was clouding in the closed arm of all tubes—heavy in glycerin and mannit, light in dextrose, and moderate in the others. Tests for ammonia with Nessler's reagent gave a positive reaction in all sugars—slight in glycerin, and moderate in the others. Titrations with phenolphthalein as indica-

determined by the colorimetric method at the end of 6 weeks. The P_H for dextrose was for maltose 5 to 5.2, for saccharose 4.6, for lactose 7, for glycerin 4.8, and for mannit 6. Controls and *Bacillus coli* Escherich showed a P_H of 4.8 throughout.

TEMPERATURE RELATIONS.—The maximum temperature for growth is above 38° C. The minimum temperature for growth is 3° . The optimum temperature for growth is 24° to 25° . The thermal death point is 48° to 50° . Tests were made by the same methods as those used for the halo organisms.

VITALITY ON CULTURE MEDIA.—The organism lives for 2 months on beef-peptone agar at room temperatures. It is nonpathogenic.

GROUP NUMBER.—The group number is 221.3333533, according to the descriptive chart of the Society of American Bacteriologists.

TECHINAL DESCRIPTION

A motile rod, with rounded ends, one polar flagellum or several, single or occasionally in short chains; average measurement 3.5 by $1.4\ \mu$; no spores, pseudozoogloae, or involution forms; facultative anaerobic. On beef-peptone agar the colonies are round, raised, smooth, lemon-yellow with entire translucent margins; deep colonies, lens-shaped and opaque. Liquefaction of gelatin begins in 2 days and is complete in 40 days. There is reduction in litmus milk in 24 hours and delayed curdling without subsequent peptonization; milk is curdled in 3 weeks without subsequent peptonization; ammonia production moderate; indol production feeble; does not produce hydrogen sulphid or other gas; no diastasic action on starch; grows moderately in Uschinsky's solution, and very copiously in Fermi's solution. Growth slight in Cohn's solution. Maximum temperature for growth is above 38° C., minimum 3° , optimum 24 to 25° , thermal death point 48° to 50° . Tolerates sodium hydroxid to below -22 Fuller's scale, and hydrochloric acid to $+20$ Fuller's scale. The optimum reaction for growth is $+5$ Fuller's scale. Gram-negative; not acid-fast; stains readily with carbol fuchsin, gentian violet, and methylene blue. Nonpathogenic to oats.

OVERWINTERING AND DISSEMINATION

There is evidence from three sources that the organism causing halo-blight winters over on the seed: (1) the presence of typical halo lesions on the glumes and lemmas of maturing spikelets (Pl. 29); (2) the early appearance of the disease on seedlings grown on soil not previously sown to oats (Pl. 28); and (3) the great difference in amount of blight in oat plots from treated and untreated seed.

(1) NATURAL AND ARTIFICIAL INFECTIONS OF SPIKELETS

In 1918 at the time the oat plants were heading out it became evident from observations of the plot of Wisconsin No. 14 and from artificial inoculation of Wisconsin No. 7 that the spikelets were also susceptible to infection with the halo organism. After the Wisconsin No. 7 plants had headed out a number of uninjured heads were sprayed with a water suspension of the organism. Another bundle of heads, bruised by drawing between the fingers, was similarly sprayed; and both were covered with glassine bags for two days. When the bags were removed infections were already appearing on the bruised spikelets as light green

discolorations on the glumes. A week after inoculation every spikelet of these panicles showed distinct typical halo lesions. Many halo lesions also appeared on the uninjured spikelets. Injured and uninjured controls sprayed with sterile water and treated in a similar way showed no halo lesions.

Early in July natural infections on the spikelets of Wisconsin No. 14 oats were observed. Flag leaves were found which showed either scattered halos or yellow halo tissue the length of the blade and sheath. Where sheaths surrounding the heads were badly haloed, every spikelet in the panicle showed infection. If there is one single lesion on a glume it appears as a typical light green to yellow halo about the point of infection. When the whole glume is infected the tissue becomes yellow and translucent between the veins. Only a few such complete infections of panicles were found. Further observations showed that infections on a small percentage of the spikelets in a panicle were not uncommon even when there were no lesions on the sheaths below. Wind and rain might easily spread the infection directly from lower leaves to panicles. Isolations from the glumes showing these lesions and from the parts inside the glumes gave typical halo organisms. Table I, which records the counts on 42 panicles in one corner of a Wisconsin No. 14 plot, will give some idea of the percentage of blighted spikelets.

TABLE I.—Number of blighted and blasted spikelets on oats naturally infected with halo-blight

Panicle No.	Number of spikelets per panicle.	Number of blighted spikelets per panicle.	Number of blasted spikelets per panicle.	Panicle No.	Number of spikelets per panicle.	Number of blighted spikelets per panicle.	Number of blasted spikelets per panicle.
1.....	58	0	6	24.....	67	2	17
2.....	54	0	11	25.....	33	0	8
3.....	77	1	5	26.....	80	0	12
4.....	66	0	14	27.....	65	4	14
5.....	55	0	22	28.....	45	0	7
6.....	55	0	12	29.....	72	0	10
7.....	55	0	17	30.....	85	12	1
8.....	60	0	11	31.....	46	0	0
9.....	42	0	14	32.....	50	25	47
10.....	64	1	13	33.....	55	0	8
11.....	90	2	16	34.....	45	0	7
12.....	51	1	9	35.....	71	29	4
13.....	61	0	22	36.....	65	0	14
14.....	79	36	25	37.....	109	5	6
15.....	18	18	23	38.....	52	0	0
16.....	87	0	4	39.....	69	8	8
17.....	50	0	13	40.....	66	1	6
18.....	39	0	21	41.....	60	3	13
19.....	54	4	34	42.....	5	21
20.....	50	5	35				
21.....	55	2	14	Total.....	2,387	165	587
22.....	62	0	23	Average.....	59+	4	14+
23.....	69	0	20	Per cent.....	6+	24+

In this case 6 per cent of the spikelets are blighted. This accords with the percentage of primary lesions usually observed on seedlings in the field. The sheaths below the panicles numbered 15, 32, and 35 were badly yellowed with halo lesions.

(2) PRIMARY LESIONS ON THE FIRST LEAVES OF SEEDLINGS

These primary lesions have been observed by the writer on more than 30 varieties of oats in Wisconsin in two different years. They may appear as halos on any part of the leaf blade, but they more often occur on the tips or margins of the leaves as shown by Plate 28.

(3) EXPERIMENTS WITH TREATED AND UNTREATED SEED

During the season of 1917 two plots of oats were planted on soil which had not previously been planted to oats. Untreated seed of each of 33 Wisconsin varieties was planted in April, and in May seed of the same 33 varieties was planted after having been soaked for 2½ hours in 1 to 320 formalin (1 pint to 40 gallons). Every one of the 33 varieties from untreated seed showed halo-blight to at least some extent, the amount decreasing as the hot weather came on. Wisconsin No. 14 showed the heaviest blighting, and Wisconsin No. 25 was also heavily spotted. Throughout the season not a single lesion was found on the 33 varieties from treated seed.

In April, 1918, three parallel plots of oats were planted on soil not previously planted to oats. Thirty-three Wisconsin varieties of untreated 1916 seed were planted in the first plot, 44 Wisconsin varieties of untreated 1917 seed were planted in the second plot, and 44 Wisconsin varieties of treated 1917 seed were planted in the third plot. Also treated seed of Wisconsin No. 14 was planted as a fourth plot on the experimental ground where oats were grown in 1917. This seed was treated by soaking for 3 hours previous to planting in 1 to 320 formalin.

Counts of infections appearing in these plots were begun just as the second leaf was coming out. On May 16, 17, and 18 primary lesions were appearing on the first leaves of plots from untreated 1916 and 1917 seed, the number of primary infections varying from less than 1 per cent to 8 per cent in each plot. These primary lesions on the 1916 plot would indicate that the organism may live for two years on the seed. No lesions were found at this time on the plot from 1917 treated seed. Counts were made again in the untreated 1917 plots on May 25, four or five days after heavy driving rains, the normal incubation period for halo lesions. Practically all the first leaves were found to be spotted, and lesions were also appearing on the upper leaves. The condition in the 1916 plot at this time was about the same and continued to parallel that of the 1917 plot. At this same time—9 days after the first appearance of the disease on the untreated plots—scattered halo spots and yellowed leaf tips were beginning to appear on the treated plot, evidently by infection from the

neighboring untreated plots, one of which was only 3 feet away. On May 24 and 25 there were more driving rains, and on the twenty-eighth the effects of these storms were evident. Secondary lesions in the untreated plots were so abundant that no attempt was made to count them. Many of the first leaves were completely yellowed and dead, and lesions on second and third leaves were so numerous that tips, margins, and even whole leaves were becoming yellowed. On varieties where infections were not so abundant the second leaves showed only scattered lesions. On the treated plot the primary lesions were still few, and there was here very striking evidence of the way in which the organism spreads about a center of infection. More or less circular spots of infected plants could be distinguished with the more heavily spotted plants in the center. The amount of infection in this treated plot gradually increased until most of the first and second leaves showed some spotting, but in none of the varieties was there more than half as much blighting as in the untreated plots. In the third treated plot, Wisconsin No. 8 showed only scattered lesions on the lower leaves and none on the upper. In the untreated plot of this variety the lower leaves were practically destroyed and the upper so badly spotted that they showed a yellow-brown color at a distance. There were similar but less marked differences in other varieties. Through June there was very little rain. The amount of blight gradually decreased until at heading time, about the first of July, very few halo lesions could be found, and the upper leaves were practically unspotted.

No halo lesions were observed on the fourth plot from Wisconsin No. 14 treated seed until about the twenty-fifth of the month, when two or three centers of infection began to appear as small yellow spots. These spread rapidly after each rain until one of them stood out as a distinct yellow spot irregularly 5 by 10 feet in diameter. The plants in this spot at heading time were 4 or 5 inches shorter than the more normal plants about them and headed out about a week later. Subsequently scattered lesions occurred on lower leaves throughout the plot and undoubtedly came either from the first infections observed or from the neighboring plots. If these primary infections had been produced by soil organisms they would probably have been much more general. Either sterilization of seed was not complete or else the infection came from the neighboring plots.

An experiment with hot-air treatment of seed gave additional proof that the organism is seed-borne. A plot from Graber oats heated to 100° C. for 30 hours showed no lesions throughout the season. There was not a single spot. The plot from untreated Graber oats showed an abundance of halo lesions through May and June. On every plant there was some spotting and many lower leaves were yellowed and dead.

This early appearance of lesions on seedlings grown on new soil, the appearance of typical halo lesions on the glumes and lemmas of the

developing spikelets from which the halo organism was isolated, and finally, the absence of the disease on plants from sufficiently treated seed all lead to the conclusion that this is a seed-borne disease.

HOSTS OTHER THAN OATS

Field observations and artificial inoculation experiments indicate that the halo-blight organism of oats does not readily infect other hosts. No halo lesions similar to those appearing on oats have been observed in the field on wheat, barley, corn, or timothy. In Jefferson and Dodge Counties, Wis., fields of oats and barley were planted so close together that the plants were intermingled at the margins. In both places the oat plants were heavily spotted with halo lesions, but even where these spotted oat leaves came in contact with the barley leaves not a halo could be found on barley. At Arlington Farm, Va., one halo lesion was found on a rye plant growing among infected oat plants, but no plates were made. The field was half oats and half rye, and although practically all the oat plants were spotted no other lesions could be found on rye.

Six different sets of inoculation experiments were carried on in the greenhouse during the winter of 1917-18 to test the pathogenicity of the halo-blight organism on wheat, rye, barley, and corn. The methods of inoculation were the same as those described above. The organisms used were stock and No. 36. The results are given in Table II.

TABLE II.—*Inoculations on other plants with halo-blight from oats*

Host.	Experiment I, stock.	Experiment II, stock.	Experiment III, stock.	Experiment IV, stock.	Experiment V, stock.	Experiment VI, No. 36.
Wheat.....	—	—	+	—	—	++
Rye.....	—	+++	—	—	+	+
Barley.....	—	+	+	—	—	++
Spelt.....	—	—	—	—	—	—
Corn.....	—	—	—	—	—	—
Oats, Wisconsin 14.....	+++	+++	+++	+++	+++	+++
Controls.....	—	—	—	—	—	—

+ Slight infection.
++ Moderate infection.

+++ Heavy infection.
— No infection.

Halo lesions were obtained on wheat in two different experiments, in the second of which the halo lesions were not so large but almost as numerous as on oats.

In three out of six experiments halo lesions were produced on rye. In the first, infection was so heavy that there was a general wilting of the leaves. Typical white organisms were isolated from these leaves which on reinoculation produced halo lesions on oats but not on rye.

Halos on barley were obtained in three out of six inoculation experiments. There were eight halos in the first experiment and two in the second. In the third experiment, six leaves had one or more halos.

Reisolation from the first halos gave typical white colonies which on subculture and reinoculation produced halos on barley and oats.

No halos were obtained on corn in four experiments, and no halos were obtained on broom corn in later experiments. Oat plants inoculated at the same time always showed abundant infection. It is evident that the halo-blight organism may attack wheat, rye, and barley to a slight extent; but in Wisconsin, at least, halo lesions in the field rarely, if ever, appear on anything but oats.

VARIETAL SUSCEPTIBILITY

All observed varieties of cultivated oats are attacked by the halo-blight to some extent. Wisconsin No. 14, both in the field and in the greenhouse, is more susceptible than any other variety and shows more lesions in later stages of development, especially on the flag leaf, rachis, and spikelets. Two varieties, Wisconsin No. 13 and Wisconsin No. 15, grown in the fields on either side of Wisconsin No. 14 during 1917, showed considerable resistance. Although leaves of Wisconsin No. 14 were badly spotted, the leaves of Wisconsin No. 13 and 15, which came in contact with them, showed little spotting. In the first plot (from 1916 untreated seed), described above, Wisconsin No. 128 showed only six primary infections, while Wisconsin No. 124 showed 169. In the second plot (from 1917 untreated seed) some varieties showed only slight secondary infections, others moderate, and some heavy infection.

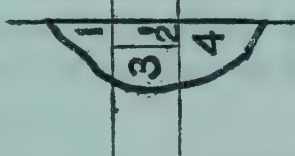
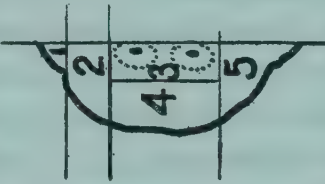

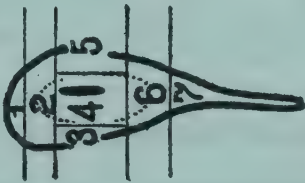
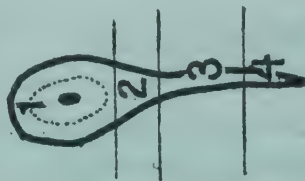
Inoculation experiments in the greenhouse also brought out differences in varietal resistance. Wisconsin No. 1, 5, and 14 were used for several experiments, Wisconsin No. 14 always showing so much heavier infection than either of the other two that Wisconsin No. 1 and 5 were no longer used. Wisconsin No. 1 showed more resistance to infection than Wisconsin No. 5.

While certain varieties are more susceptible than others under ordinary conditions and show fewer primary lesions at the beginning of the season, as above indicated, the differences are not marked in severe blight years as the season advances.

RELATION OF ORGANISM TO HALOED TISSUE

The oval outline of the halo, its rapid spread from the point of infection, and the fact that the haloed tissue remains normal, apparently, except for loss of color, have led to the conclusion that the discoloration is probably due to some diffusible substance produced by the bacteria rather than to their immediate presence. To determine whether or not the bacteria were equally distributed throughout the lesions, isolations were made from pieces of tissue cut from the centers of lesions and from points at varying distances from the center as shown in the following diagrams. Isolations were made after treatment with mercuric chlorid as described above. The distribution of bacteria throughout the halo lesions is shown in Plate 33 and Table III.

TABLE III.—Results of isolations from sections of halos at different distances from centers of lesions

	Isolation No. I.	Isolation No. II.	Isolation No. III.	Isolation No. IV.	Isolation No. V.																																							
																																												
Section No.....	<table><tr><td>1</td><td>2</td><td>3</td><td>4</td></tr><tr><td>—</td><td>+</td><td>—</td><td>—</td></tr></table>	1	2	3	4	—	+	—	—	<table><tr><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td></tr><tr><td>—</td><td>—</td><td>++</td><td>—</td><td>—</td></tr></table>	1	2	3	4	5	—	—	++	—	—	<table><tr><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td></tr><tr><td>—</td><td>—</td><td>++</td><td>—</td><td>—</td><td>—</td><td>—</td></tr></table>	1	2	3	4	5	6	7	—	—	++	—	—	—	—	<table><tr><td>1</td><td>2</td><td>3</td><td>4</td></tr><tr><td>+</td><td>—</td><td>—</td><td>—</td></tr></table>	1	2	3	4	+	—	—	—
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Growth in 48 hours.....	—	—	—	—	—																																							
Growth in 4 days.....	—	—	—	—	—																																							

+ Thin seeding of colonies. ++ Heavy seeding of colonies. — No growth.

The first two lesions used were produced by artificial inoculation. The last three were natural infections from the experimental plots. In all of the five isolations only the plates from the centers of the lesions showed any growth at all, and these plates were heavily seeded with typical white colonies. The only exception is the one colony on a plate from isolation IV, section 2. In isolation I the broth cultures from sections outside the center did not even cloud. In other isolations where broth cultures from sections outside the center clouded, subsequent plates showed that the clouding was sometimes due to the growth of the halo organism and sometimes to contamination.

The bacteria are evidently abundant only in the centers of the lesions, and if any do occur outside in the halo they are very few in number. This indicates that the discoloration of the halo-tissue is due only indirectly to the presence of bacteria, and that some enzym or toxic by-product destroys the chlorophyl. A suggestion of what this by-product might be was obtained from some plates of potato-dextrose agar on which colonies of the blight organism were growing. When colonies of the stock organism were 3 days old distinct halos appeared in the agar about the colonies as illustrated in Plate 32, B. The agar around these colonies was less translucent than that outside the halos and was distinct in outline. These halos in the agar increased in diameter from day to day, showing the concentric circles of growth illustrated in the plate and characteristic of the lesions on oat leaves. Acetic acid dropped on the agar-plate halos cleared them in a minute or two. Drops of ammonium carbonate $[(\text{NH}_4)_2\text{CO}_3]$ and ammonium chlorid $[\text{NH}_4\text{Cl}]$ on sterile plates of the same potato agar produced in a few minutes halos similar in size and appearance to those produced by the colonies of bacteria. Acetic acid also cleared these halos. Litmus was added to melted potato agar at the rate of one drop of a saturated solution to 10 cc. of the agar, and plates were poured. Streaks of stock were made across the agar as soon as it had hardened. Similar streaks on plain potato agar produced distinct halos about them in two days. In the same time the litmus potato agar had turned a distinct blue for 1 cm. or more on all sides of the growth. It seems probable, therefore, that the ammonia produced by the blight organism is responsible for the destruction of the chlorophyl and for the halolike lesions produced in the oat plants.

Stained sections of haloed leaf tissue also show bacteria only in the center of the lesion. The bacteria at first are intercellular, but later they destroy the cell walls and cause the collapse of the tissue. The collapsed tissue is evident as the dead brown centers of lesions. (See Pl. 35, C.)

COMPARISON WITH OTHER SIMILAR BACTERIAL DISEASES

Seasons of excessive rainfall and of abnormal conditions in the oat fields similar to those of 1918 have been recorded for 1890 and 1907-8. For the earlier record we are indebted to Galloway and Southworth, of the United States Department of Agriculture (1), and for the later work to Thomas F. Manns, of the Ohio Experiment Station (3).

WORK OF GALLOWAY AND SOUTHWORTH

In 1890 Galloway and Southworth (1) published a preliminary note on what they termed "a new and destructive oat disease." This disease appeared in May and June of that season and was so widespread and severe as to threaten to destroy the entire oat crop of the eastern and central States. The signs described were a browning of the tips of the lower leaves, which spread until in a short time all the leaves were dead and brown. Bacteria were found in these lesions. The account of the disease by these authors, however, is too meager to afford any basis for judgment as to whether or not it was the disease here described.

During the seasons of 1906-1909 blade-blight of oats was recorded again over a fairly wide area, and in 1907 it was so severe in some fields as to occasion a loss of from one-half to two-thirds of the crop. In 1908 the blight was threatening at one time but eventually caused little loss. The accounts of the disease from southern Canada and central and eastern States are of the same general kind. They mention a general yellowing of the lower leaves of young plants, the yellow color changing to a brown or red under weather conditions unfavorable to the organism, such as a sudden change from cool, cloudy weather to bright sunshine and higher temperature. The fields are often described as having a rusted appearance because of this reddening of the blades. The trouble was attributed to various causes—to insects, to bacteria, to fungi, and to unfavorable weather conditions.

In 1908 Dr. Erwin F. Smith discovered this disease at Arlington Farm, Va., photographed it, cut sections, and made cultures of the organism on various media, but did not publish upon it nor make any inoculations, although it is quite certain from the type of the disease and the nature of the cultures that he had the same organism here described. This was perhaps its first isolation in pure culture.

No other serious research work was undertaken until Thomas F. Manns carried on his investigations during the seasons of 1908-9 at the Ohio Experiment Station.

WORK OF THOMAS F. MANNS, 1906-1909

Manns (3) states that—

the disease manifests its presence by changes in color varying from a light yellowing, which apparently checks but little the growth of the oats, to a pronounced reddening, which in severe cases kills the blades, leaving only the younger leaves and the central axes alive.

The primary yellowing sooner or later changes to a mottled red or brown. In another place he says:

The preliminary effects of this disease is a yellowing, beginning either as small, round lesions on the blade, or as long, streak lesions extending throughout the blade or even the whole length of the culm and blade. Occasionally it begins at the tips and works back into the culm; again the upper leaves often break down through a weakened condition of the plant from defoliation below.

When lesions work back from the leaves to the culm a general yellowing and collapse of all the foliage may result. In 1909—

the disease in the majority of infected leaves began as small yellow spots on different parts of the blades. When these points of infection were numerous, the infected areas quickly became confluent, and the collapsed leaf showed a brownish mottled appearance.

These brief statements are the only references in the bulletin (exclusive of Pl. XIII) to anything at all corresponding to the lesions characteristic of the blight here described, and there is much that is contradictory. His colored figures as well as most of his text indicate an entirely different disease, but his Plate XIII shows that this halo-disease formed at least a part of the phenomenon under consideration. The distinct reddening which he describes and which he illustrates in Plates X and XI was not observed anywhere in Wisconsin even in the worst blight year, 1918. A distinct reddening of oat leaves was observed in our plots but was not due to the halo-blight. Two unsuccessful attempts were made by the writer to isolate bacteria from these reddened leaves. Manns attributes the severity of the outbreak in 1907 to the abnormally low temperatures of April, May, June, and July and to the unusual amount of rainfall during those months and gives convincing climatological data in support of his conclusion. He states that the results of artificial inoculation in the greenhouse also support this theory that cool, humid weather conditions favor the disease.

Through isolation and inoculation experiments Manns came to the conclusion that the blade-blight of oats was due to two species of bacteria living in symbiotic relations within the host tissue (*Pseudomonas avenae* Manns and *Bacillus avenae* Manns). His isolations were made by sterilizing the blades in 2 to 1,000 mercuric chlorid solutions for 1 to 1½ minutes and following this by four washings in sterile water. He states that in practically all isolations from diseased oats these two bacteria were found to be more or less abundant, and when occurring together they could be plainly seen on the agar poured plates in from 2 to 3 days. The yellow organism (*Bacillus avenae* Manns) always appeared first. As a rule, the white organism predominated.

Inoculations were made by Manns in several ways: (1) Directly from crushed leaves; (2) by hypodermic injection, using separate pure cultures of the white and the yellow organism; (3) by hypodermic injection, using the two cultures mixed (3, Pl. X); (4) by spraying mixed

cultures on injured and uninjured leaves; (5) by root inoculations without wounds, using mixtures of the two organisms; and (6) by means of grain aphids.

He reports that inoculations in the field and in the greenhouse showed that the yellow organism when used alone produced no lesions and that the white organism when used alone produced only "limited and non-typical lesions," which formed slowly, extended from $\frac{1}{2}$ to 1 inch from the point of infection, and then remained checked. When a mixture of the two organisms was used the lesions appeared in from 10 to 12 days and spread rapidly. From these results he concludes that the disease is a symbiosis, the white organism requiring the presence of the yellow organism to be actively pathogenic.

He also states that the virulence and viability of the white organism on artificial culture media depend greatly upon association with the yellow organism and that the pathogenic action of the white organism was more marked when carried over winter in mixed culture with the yellow organism than when carried over separately. After nine months in pure culture the white organism failed in several instances to grow.

Manns states that endospores occur. These were stained with hot carbol fuchsin from 2-months-old cultures. The figure of these spores in his Plate IX is too indistinct to be of any value in verifying his statement.

His white organism is described as a short motile rod with polar flagella. These are three to five times the length of the rods in his Plate IX, fig. 4, and one to six times those in his text figure No. 1. The rods measure in the majority of cases 0.75 by 1.5 μ . They are rarely in chains of three to four.

The thermal death point is 60° C. The optimum temperature is 20° to 30°. He states that his organism is pathogenic on oats, corn, timothy, barley, wheat, and bluegrass.

The group number for his white organism is given as 111.2223032. Manns' yellow organism is a bacillus with the group number 222.2223532.

Manns suggests the probability of the organism's wintering over in the soil and so being distributed to the leaves by spattering rains. He states that there is no doubt that on seedlings lesions sometimes start on the roots or on that part of the stem in contact with the soil. He does not describe these lesions. The possibility that the disease is seed-borne is not mentioned.

Manns' descriptions of individual lesions are so meager and his descriptions of general signs so inclusive as to lead to grave doubt about his having worked with a single bacterial disease. There is no doubt, however, that he sometimes had typical halo-blight lesions, because of his Plate XIII, but with this exception there is no conclusive evidence from either his text or figures that he had this disease under observation; and the

result of his inoculations as indicated on his colored plates is quite contradictory.

The chief differences between the two white organisms *Pseudomonas avenae* Manns and *Bacterium coronafaciens*, n. sp., are summarized below:

PSEUDOMONAS AVENAE MANNs.

1. Produces typical blight lesions only when used with *Bacillus avenae* Manns (a yellow organism).
 2. Spreads throughout the lesion when used alone.
 3. Virulence and viability on artificial media dependent upon association with *Bacillus avenae* Manns.
 4. Viability and virulence greatly reduced by a number of transfers.
 5. Growth feeble on artificial media. (See 3, Pl. VIII, fig. 3.)
 6. Liquefaction of gelatin stabs begins in 7 to 12 days.
 7. Pitting of gelatin colonies begins in 7 days.
 8. Visible growth in broth in 3 days.
 9. Manns does not record browning of broth or other media.
 10. Milk not coagulated in 30 days.
 11. Acid to litmus milk.
 12. No reduction of litmus milk recorded.
 13. Strictly aerobic.
 14. No ammonia produced.
 15. Nitrates reduced.
 16. Limits of growth, -5 to +15.
 17. Thermal death point 60° C.
 18. Internal structure of agar colonies amorphous.
 19. In hanging drop there are few motile organisms.
 20. Growth viscid on agar.
 21. Produces clostridium forms in one week on nutrient glucose agar.
 22. Produces endospores.
 23. Does not form long chains.
 24. Shorter and thicker than *Bacterium coronafaciens*. Average size 0.75 by 1.5 μ .
 25. Lives over in the soil.
 26. Pathogenic on oats, corn, timothy, barley, wheat, and bluegrass.
- Group number 111.2223032.

BACTERIUM CORONAFACIENS, N. SP.

1. Produces typical halo-blight lesions when used in pure culture.
 2. Found only about the point of infection and not throughout the halo.
 3. Virulence and viability not dependent on another organism.
 4. Viability and virulence not reduced by transfer.
 5. Growth abundant on artificial media. (See Pl. 30, A, B, a, b.)
 6. Liquefaction begins in 3 days.
 7. Pitting begins in 3 days.
 8. Visible growth in 1 day.
 9. Broth and other media turned brown.
 10. Milk usually coagulated in 5 to 7 days.
 11. Alkaline to litmus milk.
 12. Litmus milk reduced.
 13. Facultative anaerobic.
 14. Ammonia produced.
 15. Nitrates not reduced.
 16. Limits of growth, -18 to +27.
 17. Thermal death point 47° to 48° C.
 18. Internal structure of agar colonies not amorphous. (See Pl. 31.)
 19. Active motile organisms in hanging drop.
 20. Growth butyrous.
 21. No clostridium forms observed in any medium.
 22. Does not produce endospores.
 23. Forms chains and long filaments.
 24. Average size 0.65 by 2.3 μ .
 25. Lives over winter on the seed.
 26. Pathogenic on oats, barley, wheat, and rye.
- Group number 221.2323023

A bacterial disease producing lesions similar to those of the halo-blight of oats has been described from tobacco (10). The lesions are similar to

the halos of oats in that they form "circular chlorotic areas" 2 to 3 cm. in diameter with minute brown centers. The oat lesions, however, have no water-soaked borders, and the affected tissues do not fall out as in tobacco wildfire. A white organism has been isolated from these lesions which differs from the halo-blight organism in the points mentioned below:

HALO-BLIGHT ORGANISM.	TOBACCO ORGANISM.
One to several polar flagella.	One polar flagellum.
Single to long chains.	Single to chains of five elements.
2.3 by 0.65 μ .	3.3 by 1.2 μ .
Capsules.	No capsules.
Odor in agar stroke.	No odor in agar stroke.
Casein not precipitated in litmus milk.	Casein precipitated in litmus milk.
Ammonia produced.	Ammonia not produced.
Thermal death point 47° to 48° C.	Thermal death point 65° C.

The halo lesion so characteristic of this oat disease does not occur in the blackchaff disease of wheat (6-9) or the bacterial blight of barley (2), while the oat disease lacks the translucent water-soaked stripes of these diseases as well as the exudate so abundant in both. R. H. Rosen has recently published a preliminary note on a bacterial disease of foxtail (4), which he thinks may be similar to the halo-blight of oats. His description of lesions as dark brown spots or streaks, however, makes it probable that if it is similar to either bacterial disease of oats it would resemble stripe-blight rather than halo-blight. The writer has not observed halo lesions on foxtail and in two sets of field inoculations has obtained no infections on foxtail with the halo organism.

CONTROL MEASURES

The evidence that the halo-blight of oats is seed-borne seems conclusive. However, no practical method of seed treatment has, as yet, been found which will entirely control the disease. Treatment with formalin for smut controls halo-blight to a marked extent but not entirely. In 1917, treated seed of 33 Wisconsin varieties did not show a halo lesion throughout the season, while the same untreated varieties all showed some halo-blight. In 1918, 44 treated varieties of Wisconsin oats developed primary lesions which, however, were later and fewer than on the same untreated varieties. Even when the blight was most severe it was only about half as heavy in the treated plots as in the untreated. The plot from Wisconsin No. 14 treated seed showed very few primary lesions and little secondary spotting except in patches about these primary lesions. This would indicate that soaking for three hours in 1 to 320 formalin kills many but not all of the organisms on the seed. In Jefferson County, Wis., where most of the seed was treated for smut, the blight during the 1918 season was much less abundant than in Dodge County, where seed treatment was not general.

Another method of seed treatment is being developed at Wisconsin which in 1918 entirely controlled halo-blight. The treated seed was heated in a gas oven at 100° C. for 30 hours. The plot from this treated seed did not show a single halo lesion even during the time when other oats were most severely attacked. The plot from untreated seed of the same variety showed primary infections on 10 per cent of the plants and 100 per cent secondary infections on the lower leaves during May and the first two weeks in June. While oats in good condition successfully withstand this treatment of 30 hours at 100° C., a similar treatment for a shorter period would perhaps be just as effective. The commercial application of this treatment has not as yet been worked out.

SUMMARY

A bacterial disease known as halo-blight was unusually severe in its attack on oats throughout Wisconsin during the 1918 season, and reports of a similar disease were received from southern Minnesota, Iowa, northern Illinois, and Indiana. Such epidemics occur under particularly favorable weather conditions, disappearing with the advent of weather conditions more favorable to the development of the host plant.

Typical lesions of halo-blight are characterized by halolike margins of chlorotic tissue about a center of dead tissue.

Isolations from these lesions have constantly given a typical white organism. Yellow organisms also appear from isolations when the surface of the tissue has not been sterilized.

Inoculation experiments have shown conclusively that the white organism alone is responsible for the production of typical lesions. The yellow organism is evidently a surface saprophyte.

Since few if any organisms are found outside the central infection area, the halo is thought to be due to a diffusible substance, probably ammonia.

The organisms live over winter on the seed, producing primary lesions on the first leaves of seedlings. From these lesions the organisms are carried to other leaves by wind and rain.

It seems probable that the percentage of blasting on oat panicles varies with the severity of the halo-blight from season to season. This blasting seems to be due to the same unfavorable weather conditions which favor the development of the bacterial blight rather than to the disease itself.

Halo-blight lesions from natural infections have never been observed on any hosts except oats and rye. Artificial inoculations show that the halo organism may be slightly pathogenic on wheat, rye, and barley.

When the halo-blight is not too severe, different varieties of oats show differences in susceptibility to the disease.

The organism isolated and described by the writer has the group number 221.2323023. No other white organism used by the writer has produced anything similar to the halo lesions. Other white organisms have in fact produced no lesions on oats. Three strains of softrot organisms with internal markings very much like those of oat colonies have been used, and also the white organism, *Bacterium atrofaciens* McC., which produces lesions on wheat. The name of *Bacterium coronafaciens* n. sp. is suggested for this white halo-producing organism.

Treatment with 1 to 320 formalin, as for smut, checks but does not entirely control the disease. A hot-air treatment for 30 hours at 100° C. does control the blight.

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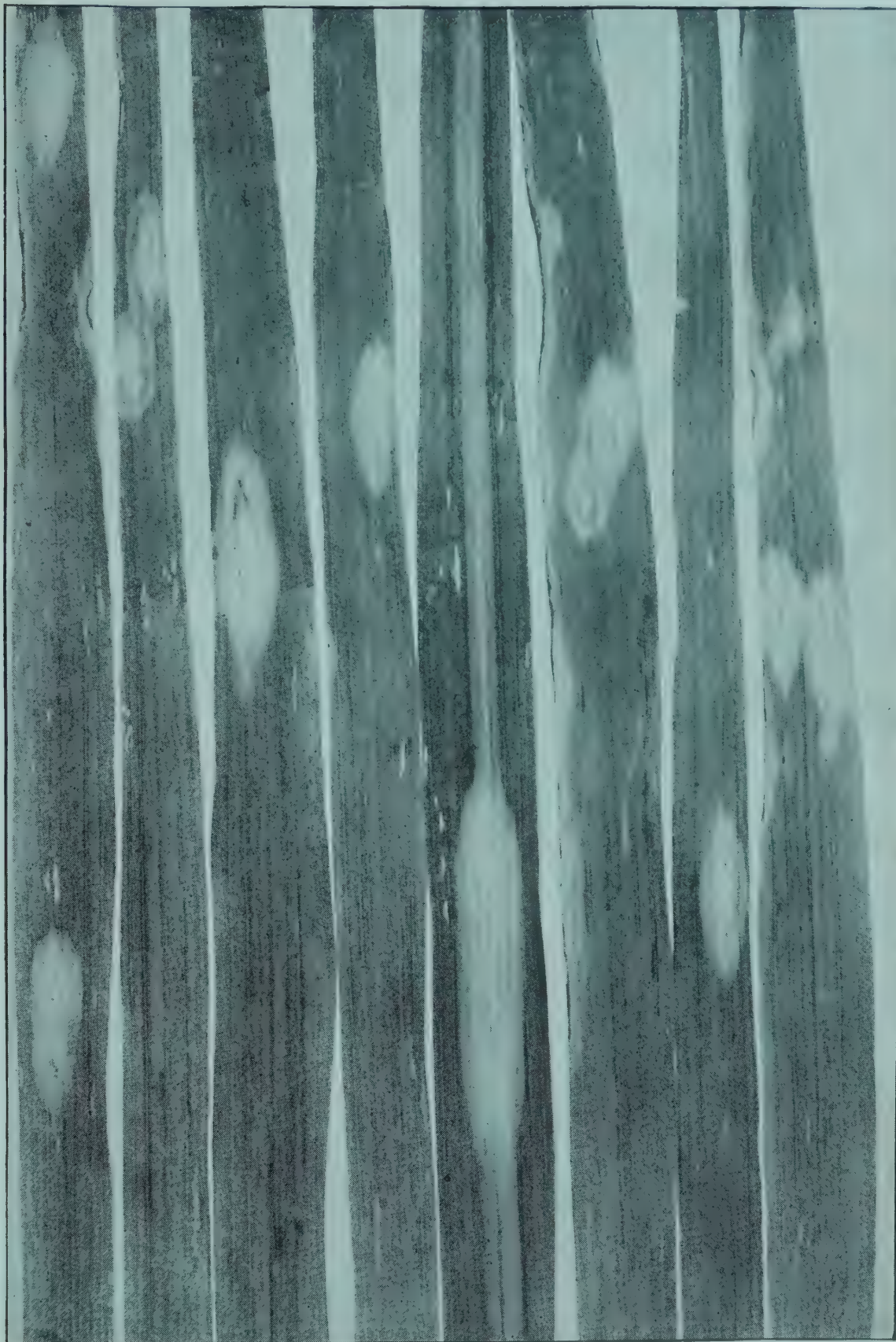


PLATE C

Halo lesions on flag leaves of Wisconsin No. 14 oats. Natural infections from Hill Farm, Madison, Wis. Photographed June, 1917.

PLATE 26

Typical isolated halo lesions. Natural infection on Graber oats. Photographed June 24, 1918. Natural size.



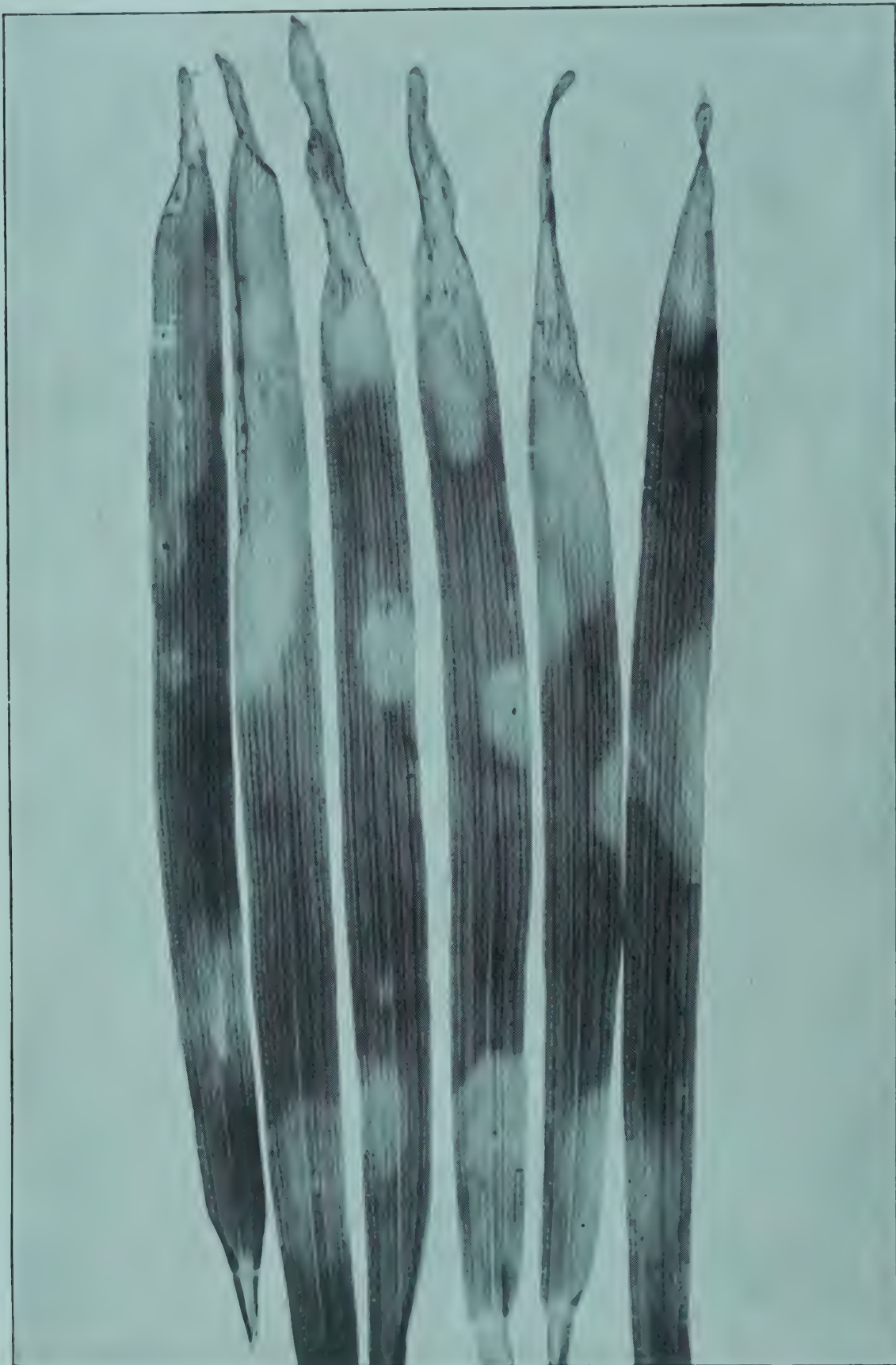


PLATE 27

Halo lesions on Wisconsin No. 14 oats produced by spraying with a water suspension of the stock organism May 26, 1917. Photographed May 31, 1917.

PLATE 28

Infection from untreated 1916 seed of Wisconsin No. 124 oats. Planted April 24, 1918. Photographed May 17, 1918.

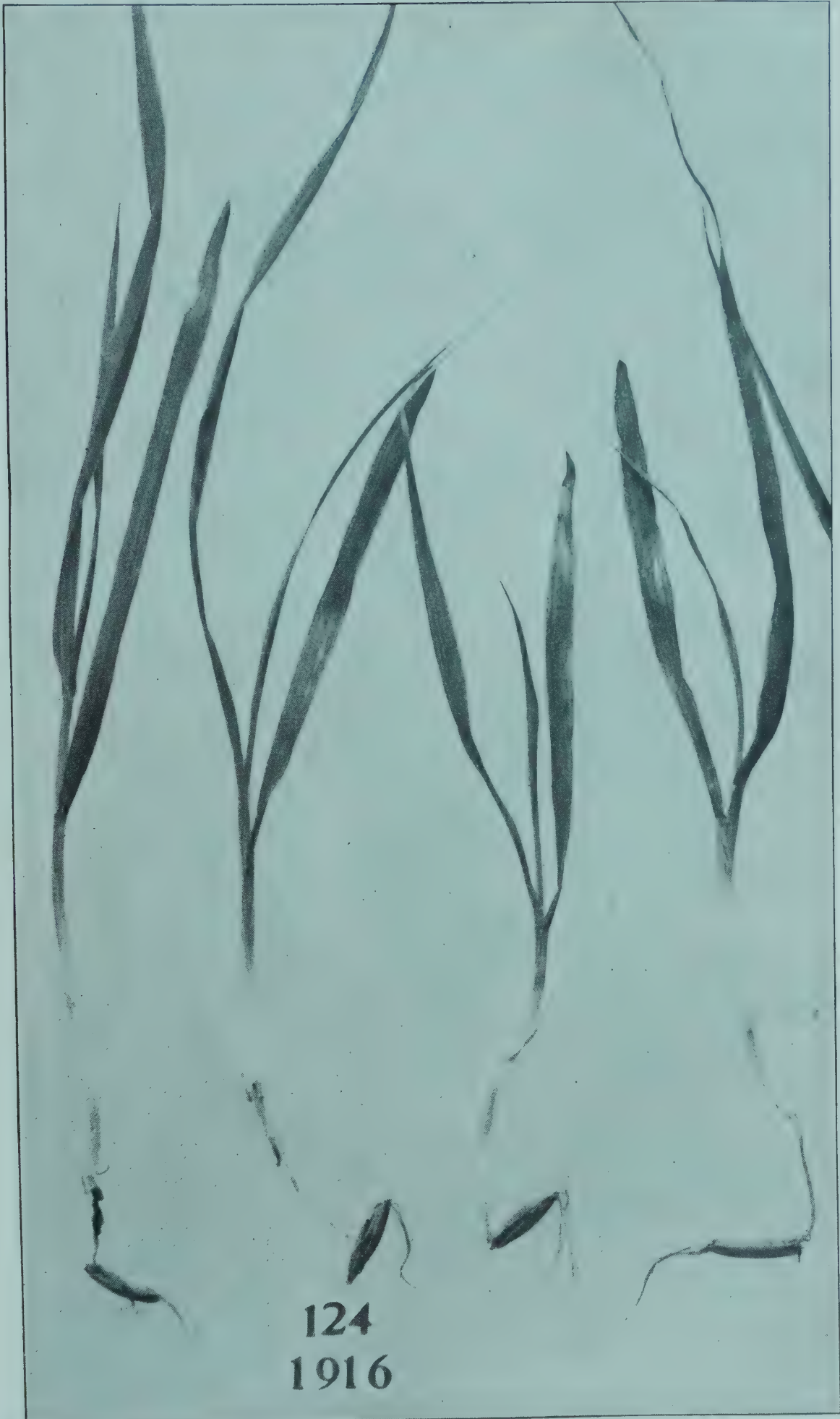




PLATE 29

Spikelets of Wisconsin No. 14 oats:

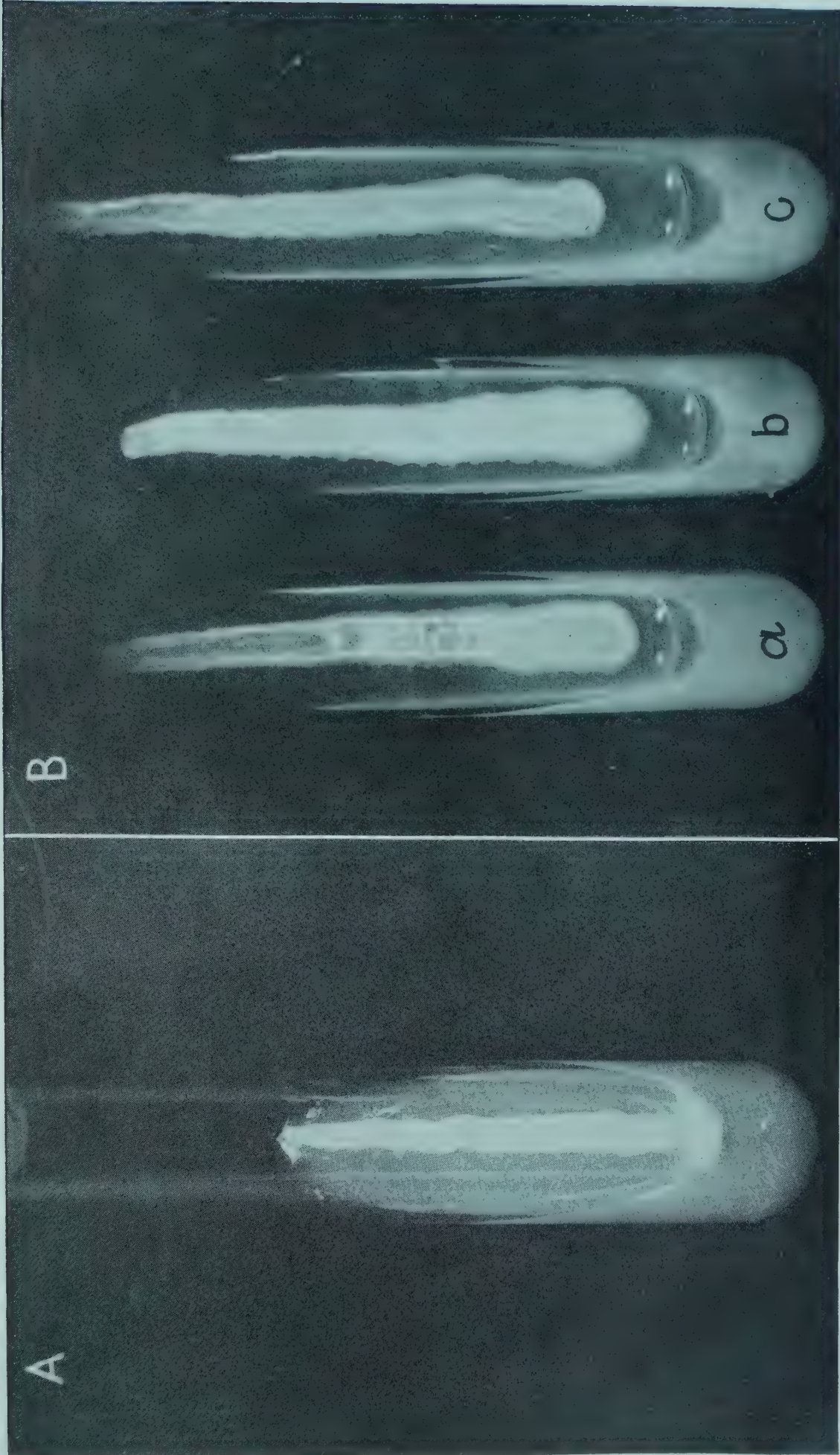
A.—Left and center spikelets show natural infection with halo-blight. Tips yellowed and translucent. Spikelet at right normal, unspotted. Photographed July 17, 1918.

B.—Upper spikelet shows typical isolated halo lesion near base. Lower spikelet normal, unspotted.

PLATE 30

A.—Two per cent +5 glucose Difco peptone beef bouillon agar slant of No. 36. Three-day colony. Photographed August 29, 1919. Natural size.

B.—Two per cent potato-dextrose agar slants. *a*, No. 36, white culture, consistency butyrous; *b*, stock, white culture, consistency of boiled starch; *c*, No. 39a, yellow culture.



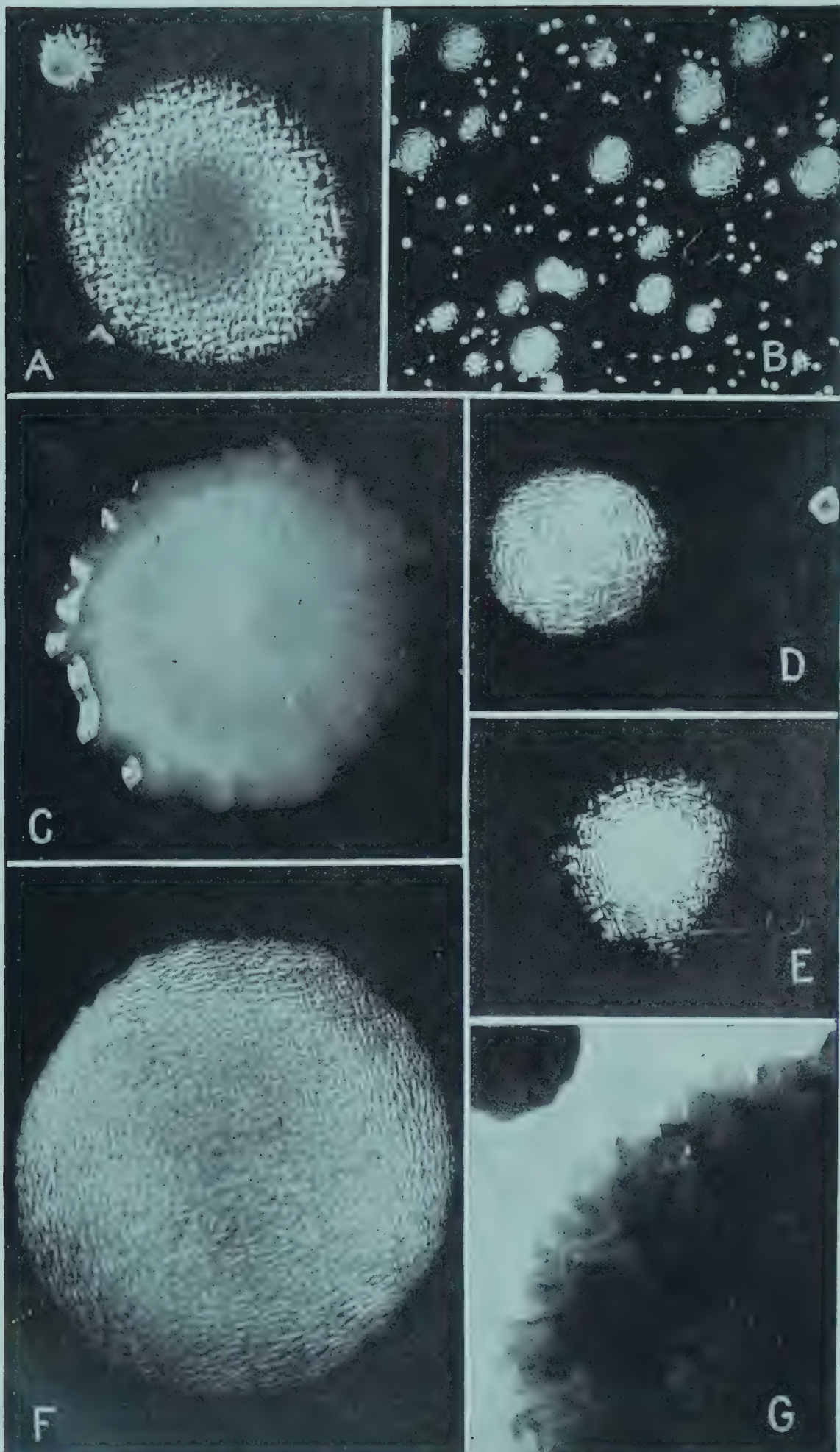


PLATE 31

A.—Three-day colony of stock on 2 per cent dextrose-potato agar. Photographed February 17, 1919, by oblique transmitted light. $\times 10$.

B.—Two-day colonies of stock on + 10 beef-peptone agar. Photographed March 26, 1919, by oblique transmitted light. $\times 10$.

C.—Five-day colony of stock on potato-dextrose agar. Colony of boiled starch consistency. Photographed January, 1918, by reflected light. $\times 7$.

D.—Five-day colony of No. 36 on + 10 beef-peptone agar. Photographed October 1, 1918, by oblique transmitted light. $\times 10$.

E.—Three-day colony of stock on 2 per cent glucose Difco peptone beef bouillon agar. Photographed October 7, 1919, by oblique transmitted light. $\times 10$.

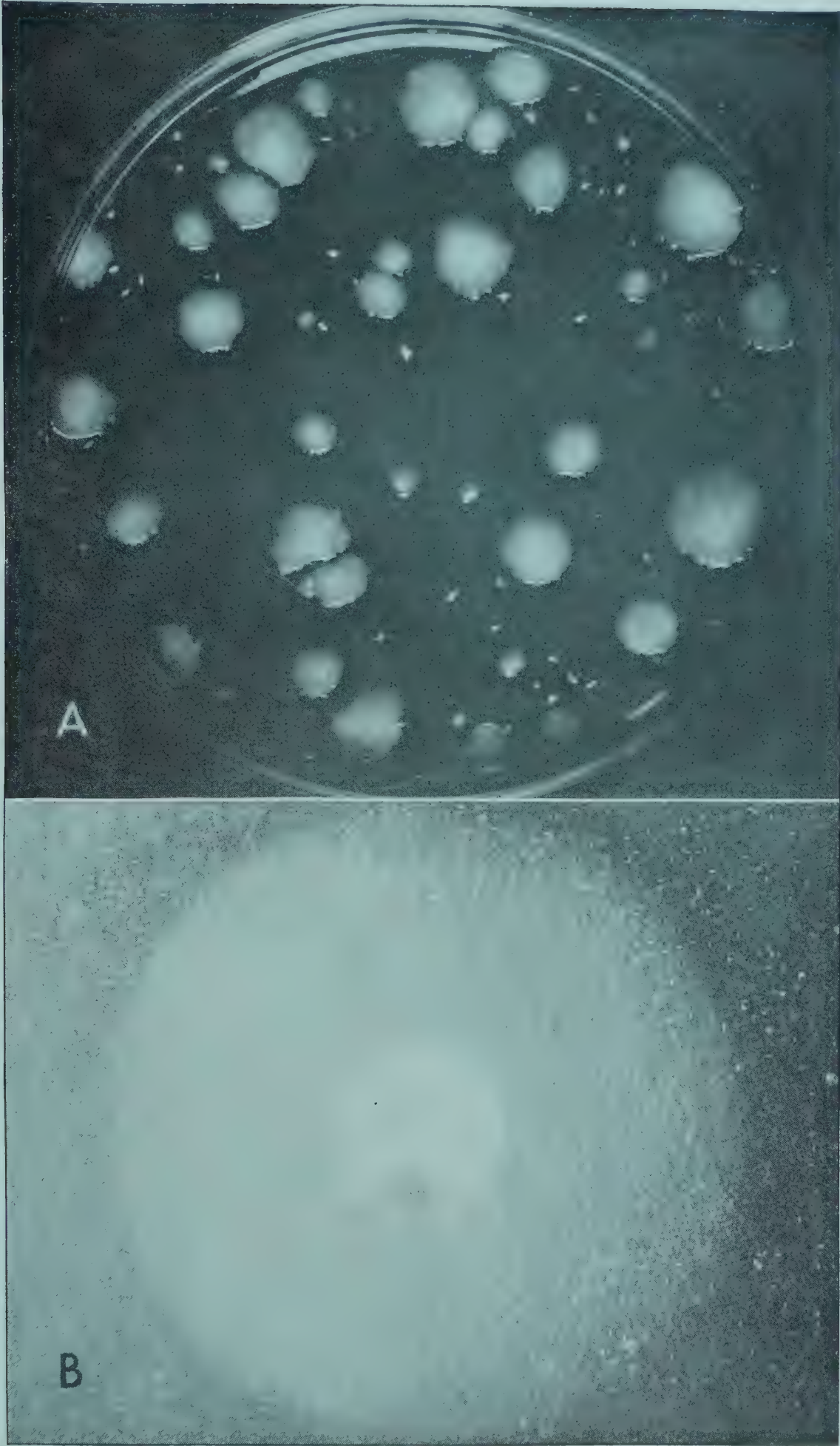
F.—Seven-day colony of stock on + 15 beef-peptone agar. Photographed March 31, 1919, by oblique transmitted light. $\times 10$.

G.—Margin of 3-day colony of stock on + 15 gelatin. Photographed September 30, 1919. $\times 75$.

PLATE 32

A.—Five-day colonies of stock on potato-dextrose agar. Colonies of boiled starch consistency. (For single colony see Pl. 31, C.) Photographed by reflected light. Natural size.

B.—Three-day colony of stock on potato-dextrose agar. Halo about colony. Photographed February 17, 1919, by oblique transmitted light. $\times 5$.



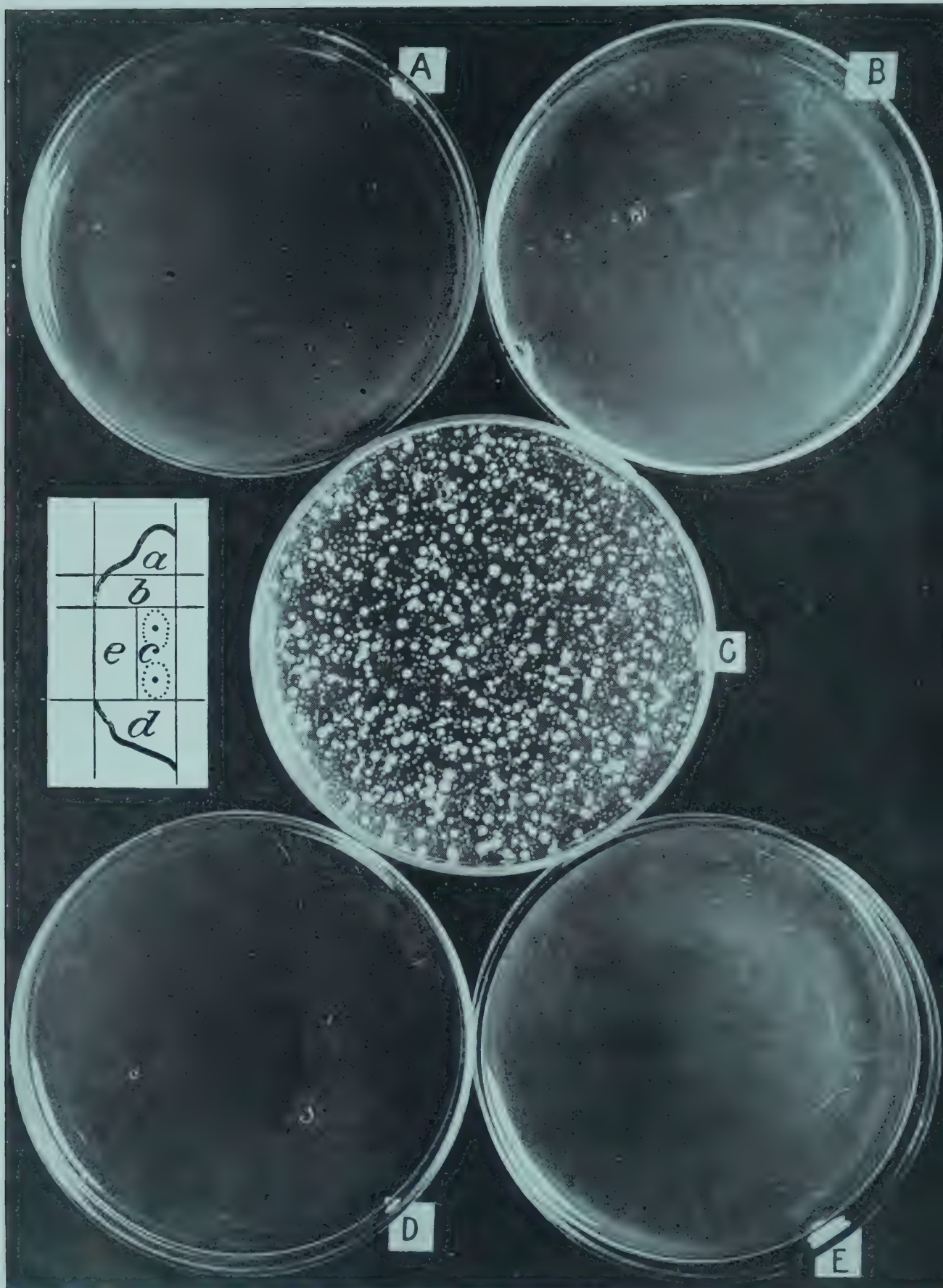


PLATE 33

Isolations from sections of halo lesion. The lesion was from an artificial inoculation with oat stock organism made February 25, 1918. Isolations were made March 13, 1918, on potato agar. The sections were dipped in alcohol and then submerged for one minute in 1 to 1,000 mercuric chlorid. The plate from section *c* is the only one showing colonies of bacteria.

A.—Poured plate of isolation from section *a* of lesion.

B.—Poured plate of isolation from section *b* of lesion.

C.—Poured plate of isolation from section *c* of lesion.

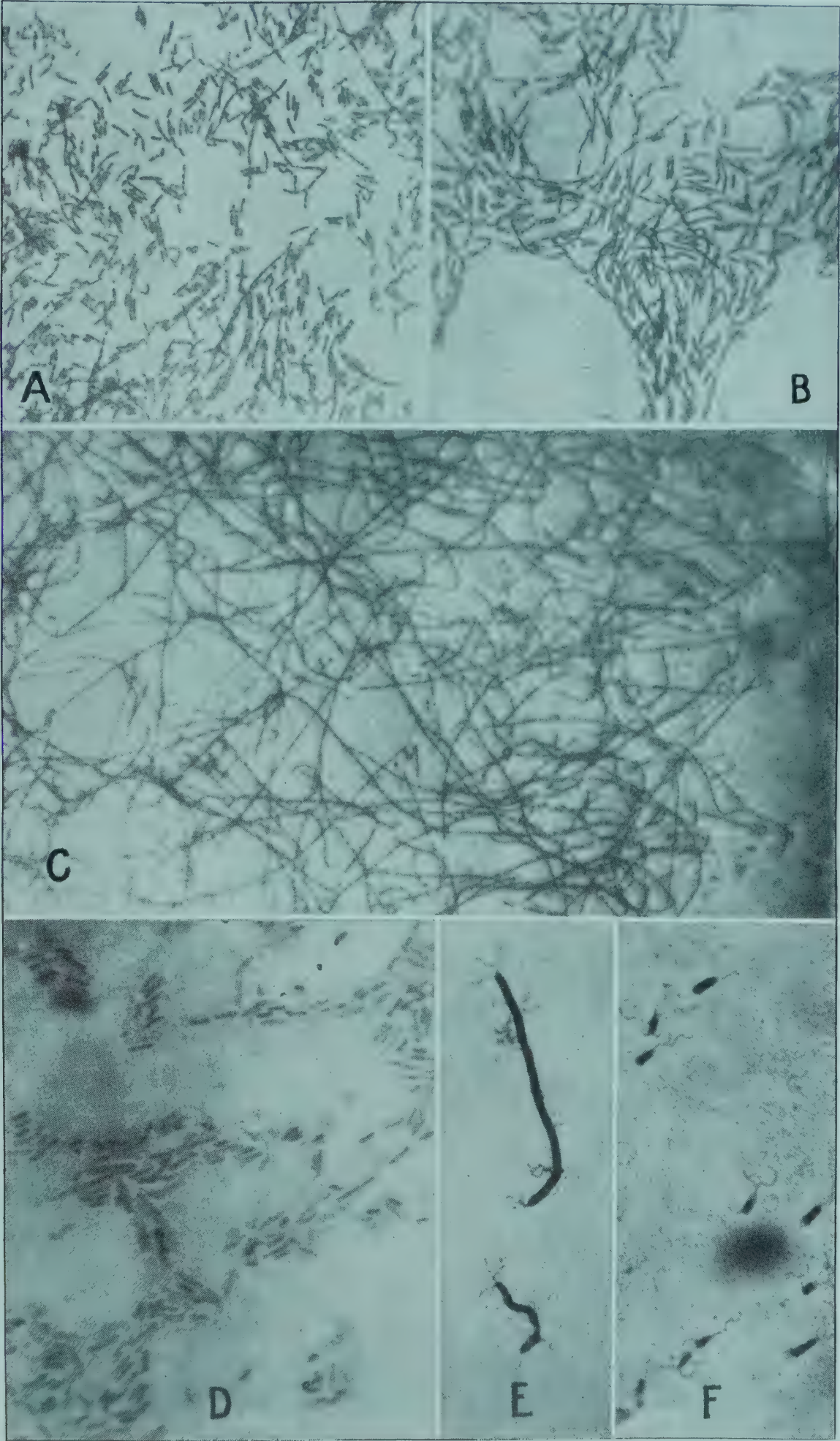
D.—Poured plate of isolation from section *d* of lesion.

E.—Poured plate of isolation from section *e* of lesion.

Photographed March 15, 1918.

PLATE 34

- A.—No. 36 from 24-hour potato-dextrose agar slant; carbol fuchsin stain. $\times 620$.
- B.—Stock from 24-hour potato-dextrose agar; Ribbert's capsule stain. $\times 620$.
- C.—Stock from 4-day potato-dextrose agar; carbol fuchsin stain, showing long chains. $\times 620$.
- D.—Stock from 3-day potato-dextrose agar; Ribbert's capsule stain. $\times 1,550$.
- E.—Stock from 1-day + 15 beef-peptone agar; Van Ermengem stain. $\times 1,550$.
- F.—No. 36 from 1-day + 5 beef-peptone agar; Caesar-Gil stain. $\times 1,550$.



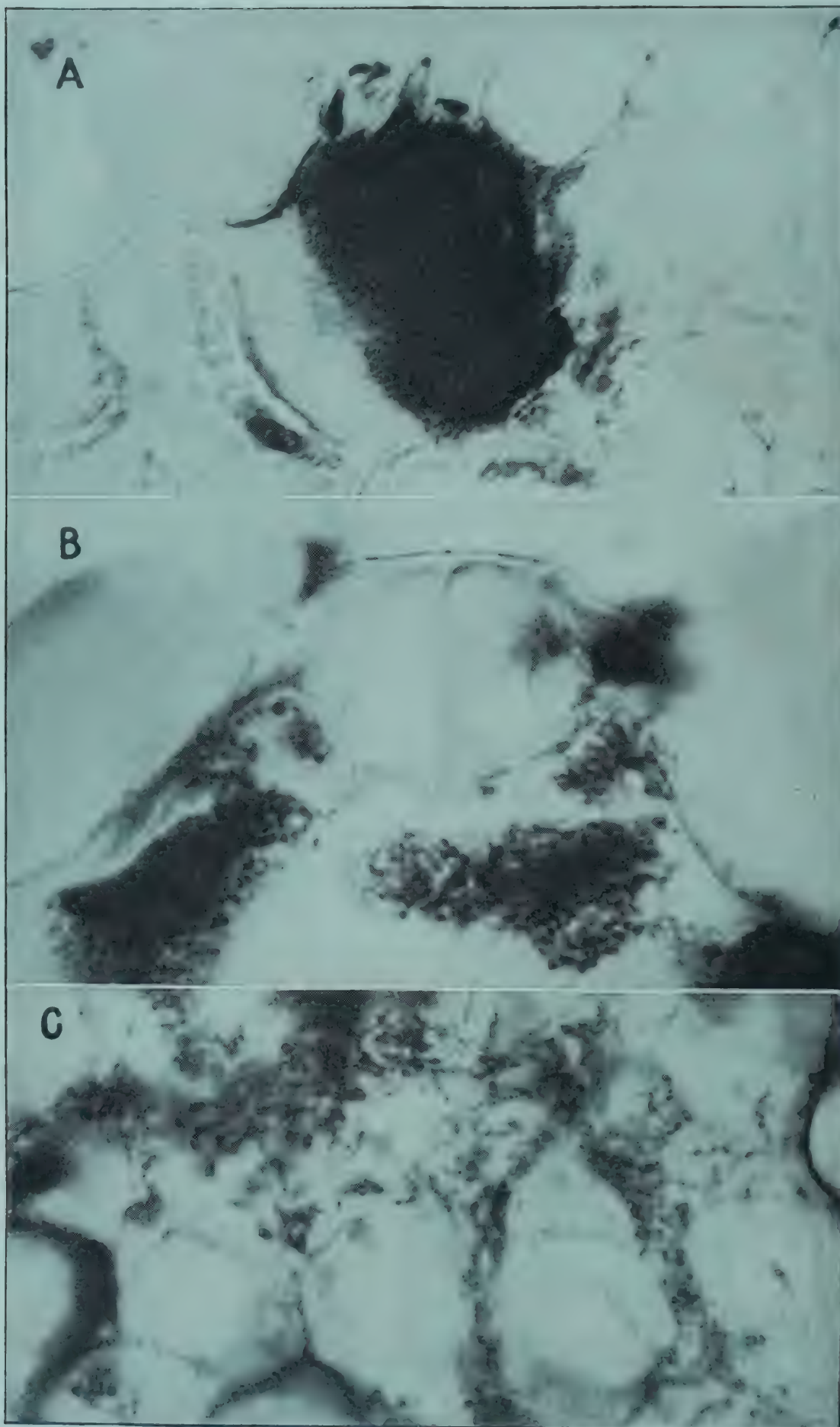


PLATE 35

Sections of oat leaves through halo lesions, showing bacteria in the tissues. Fixed in Gilson's fixative and stained with carbol fuchsin.

A.—Bacteria in substomatal cavity, showing method of entrance of bacteria into the leaf tissue. Cut $15\ \mu$ thick. $\times 700$.

B.—Bacteria in substomatal cavity. Cut $15\ \mu$ thick. $\times 1,650$.

C.—Section of older lesion, showing bacteria between the cells. In the upper part of this section the tissue is disintegrating at about the point of infection.

Photographed August 26, 1919. $\times 1,550$.

INFLUENCE OF FERMENTATION ON THE STARCH CONTENT OF EXPERIMENTAL SILAGE

By ARTHUR W. DOX, *Chief in Chemistry*, and LESTER YODER, *Assistant Chemist*, Iowa
Agricultural Experiment Station

INTRODUCTION

It is not definitely known, though sometimes assumed, that polysaccharoses undergo changes during the formation of silage from green corn. The work reported in this paper was undertaken to determine any changes the starch might undergo together with the nature of these changes and their relation to other important reactions occurring in silage fermentation.

PREVIOUS INVESTIGATIONS

Results of acidity, alcohol, and sugar determinations have been reported by Dox and Neidig (5, 6)¹ and Lamb (9) in investigations extending over initial fermentation periods of 30 days and less. Acids and alcohol are rapidly formed at the expense of the sugar, the rate and extent of the changes depending largely upon the nature of the corn.

Babcock and Russell (2), Hart and Willaman (8), and Esten and Mason (7) have made important contributions bearing upon the common changes occurring in silage.

No investigations concerning the starch of corn silage have been reported. Statements are made in an article by E. J. Russell (13) of the Rothamsted Station to the effect that bacteria are present which attack the less resistant celluloses and that the disappearance of some less resistant celluloses is a characteristic silage change.

EXPERIMENTAL METHODS

The plan followed by the writers provided for the examination of normal experimental silage at various stages of fermentation. Field corn still green, dented, and about at its glazing stage, cut at about 9 a. m., was taken in bags from the farm silage cutter, brought to the laboratory, and further chopped in the laboratory feed cutter. The chopped silage was then thoroughly mixed, and 2.5 kgm. were packed uniformly into each of 10 wide-mouthed glass jars at 3 p. m. of the same day. The jars were then covered, sealed with paraffin, provided with a valve escape for gases, placed in a large box, and well insulated from exterior temperature influences. On the first day the temperature of the silage rose to 29° C. It remained there for two days, then dropped gradually to room temperature by the seventh day.

¹ Reference is made by number (*italic*) to "Literature cited," pp. 178-179.

Immediately after the jars were filled a sample of the original chopped corn was examined. Thereafter a jar of the silage was opened and examined on the second, fourth, sixth, eighth, twelfth, eighteenth, twenty-ninth, forty-fourth, sixty-sixth, and ninetieth days. Determinations were made for moisture, total acidity, alcohol, total sugar, and starch. As a matter of expediency, qualitative tests were made for the transitional products of starch hydrolysis, namely, soluble starch and dextrins.

Although similar data upon total acidity, alcohol, and sugars have been published, this work was repeated because the amount of these products varies so widely in silage made of corn from different sources that correlation with starch changes in this silage would be impracticable. Furthermore, the determinations serve to show that fermentation was normal; and when arranged in series to show changes beyond the first month, they may furnish information not available hitherto.

METHODS OF ANALYSIS

Determinations of constituents soluble in water were made in centrifuged and filtered juice pressed from 2 kgm. of the silage with an hydraulic press.

MOISTURE.—Four hundred gm. of silage were oven-dried at 100° C. to constant weight.

TOTAL ACIDITY.—Twenty-five cc. of silage juice were diluted to volume in a 100-cc. graduated flask with neutral 95 per cent alcohol, mixed and filtered. A 50-cc. aliquot was pipetted into about 300 cc. of neutralized distilled water and titrated with *N/10* barium hydroxid and phenolphthalein indicator.

ALCOHOL.—The aeration method of Dox and Lamb (4) was used. Twenty-five cc. of silage juice in a 100-cc. cylinder, saturated with ammonium sulphate, were aerated by aspirating air for 24 hours through the alcoholic solution from a dichromate oxidizing solution and through two cylinders, the first containing about 18 cc. and the second about 8 cc. concentrated sulphuric acid. The sulphuric-acid alcohol solution was then transferred to a 500-cc. distilling flask with water free from carbon dioxid, and after the addition of 5 gm. sodium dichromate, it was distilled through a Hopkins trap. The distillate was titrated and the weight of alcohol calculated from its acetic-acid equivalent.

SUGARS.—Determinations were made in preserved samples of the juice. Seventy-five cc. of silage juice were neutralized in a 150-cc. graduated flask with calcium carbonate and made up to volume with absolute alcohol and stored. Of this mixture 100 cc. were diluted to volume in a 250-cc. graduated flask with 95 per cent alcohol. From this point the alcohol extraction method published by Bryan, Given, and Straughn (3) was followed, and sugar was determined by the copper method of Munson and Walker.

STARCH.—After much preliminary work it was found that even by grinding the undried silage in the best grinder available for the purpose a degree of fineness could not be obtained which would give as high results as those secured by drying the silage and then reducing it with a Merker mill till it would pass through a 30-mesh sieve. It was also found that the polarimetric method of Lintner as modified by Porst and Crown (11) gave dependable and highly accurate results.

Five gm. of the powdered silage prepared from the residue of the moisture determination were mixed with 20 cc. of water in a mortar and cooled in ice water. To this there were added 40 cc. concentrated hydrochloric acid previously cooled. The mixture was kept at 20° C. for one-half hour. The contents of the mortar were then transferred to a 200-cc. graduated flask with hydrochloric acid of specific gravity 1.125, and 8 cc. of 4 per cent phosphotungstic acid were added. At this point it was found necessary to add charcoal (norite) decolorizer. The mixture in the flask was made up to the mark at 20° with hydrochloric acid of specific gravity 1.125 and kept at 20° for one-half hour. It was then filtered and exactly 15 minutes after filtering (1 hour and 15 minutes after the addition of the 40 cc. concentrated acid) the reading was taken at 20°. From the rotation of 5 gm. pure starch the percentage of starch in the silage was calculated.

Corrections for the zero reading and for optically active substances other than starch were made as follows: A 5-gm. sample was placed in a 200-cc. graduated flask; 100 cc. of 50 per cent alcohol were added; and the whole was boiled for one hour on the steam bath, then cooled and made up to volume with 95 per cent alcohol, mixed and filtered. A 100-cc. portion of the filtrate was evaporated almost to dryness, diluted to about 20 cc. with water, and cooled. The modified Lintner procedure was then followed as outlined above.

QUALITATIVE TESTS.—(1) For soluble starch the test was made by applying the ordinary starch test with iodine to the centrifuged and filtered juice. (2) The dextrin test consisted in adding a sufficient amount of warm saturated solution of barium hydroxid to produce a flocculent precipitate, quickly cooling and filtering, then precipitating the barium in the filtrate with carbon dioxide, refiltering, and adding a slight excess of hydrochloric acid and dilute iodine solution. The presence of dextrans was shown by a red coloration above that of the iodine solution.

EXPERIMENTAL RESULTS

The results were all calculated to the wet basis of the original silage. No correction is made for the specific gravity of the silage juice, since for all practical purposes this error is entirely negligible.

The data for acidity, alcohol, and sugar given in Table I are similar to data obtained by others. A discussion of these is not an object of

this paper except as they relate to starch changes. These results when compared with similar results obtained by previous investigators with silage produced in silos indicated that the silage was normal in every respect.

The silage of each jar examined had a characteristic silage aroma and was free from molds. The fermentation had passed its maximum activity by the eighth day and continued after the first month at a barely appreciable rate.

TABLE I.—*Analysis of experimental silage at different stages of fermentation*

Age of silage.	Moisture.	Total acidity, ^a	Ethyl alcohol.	Total sugar, as invert.	Starch.
<i>Days.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
0.25.....	65.56	38.0	0.01	2.94	10.67
2.....	65.87	126.5	.12	1.84	10.30
4.....	66.75	211.5	.11	.82	9.93
6.....	66.00	262.4	.16	.47	10.41
8.....	66.38	266.6	.10	.55	9.54
12.....	66.62	279.8	.15	.50	9.62
18.....	65.63	288.8	.19	.42	10.01
29.....	66.38	294.7	.26	.32	9.87
44.....	65.50	291.9	.28	.33	10.77
66.....	67.12	316.8	.36	.48	9.54
90.....	66.00	298.3	.39	.48	10.10

^a Expressed in cubic centimeters of *N*/10 barium hydroxid required to neutralize 100 gm. of silage.

MOISTURE.—Factors usually affecting the moisture content of silage are seepage and excessive respiration accompanied by decomposition of sugar or higher carbohydrates, as studied by Appleman (1) in picked sweetcorn, seepage resulting in a decrease of moisture, and respiration resulting in an increase. Moisture loss by seepage occurs only in silage having an abnormally high water content. The moisture content of the silage in this case was normal and remained constant at about 66 per cent. No excessive decomposition of carbohydrates by respiration is therefore indicated.

TOTAL ACIDITY.—The silage solution, the medium in which fermentation takes place and which is in contact with the silage starch granules, reached a *N*/0.4 concentration by the eighth day and almost *N*/0.5 by the sixty-sixth day. Most of this acidity is due to lactic and acetic acids which are little dissociated and leave after all a small concentration of acid. To bring starch into solution in an acid mixture more or less drastic treatment is necessary; strong acids must be used and their dilute solutions must be heated.

ALCOHOLS.—The formation of alcohol in silage is due to both bacterial growth and enzymic action, their combined effects upon the alcohol production being such that alcohol is not present in uniform quantities

throughout the fermentation period. Appreciable increases in alcohol occurred up to the third month, finally reaching a concentration of 0.39 per cent. That there was no marked maximum production of alcohol at any time was due probably at first to oxidation to acetic acid and later to esterification.

SUGAR.—A maximum loss of sugar from the silage occurred by the sixth day, when the sugar had dropped from 2.94 to 0.47 per cent. After the eighth day the results were quite constant, indicating exhaustion of sugar and the presence of reducing substances which were unfermentable under the conditions existing in this silage. Unless the rate of fermentation equals the rate of formation of sugar no formation of sugar from higher carbohydrates is indicated after the eighth day.

SOLUBLE STARCH AND DEXTRINS.—At no time were positive tests obtained for these products in the silage juice. If they are transitional in the decomposition of starch in the silage, they are so rapidly changed to simpler decomposition products that they are never present in reacting quantities even in green corn. Only in cases of rapid gelatinization of relatively large quantities of starch would tests for these constituents be positive in a medium like that existing in silage. Their absence indicates that the insolubility of the silage starch is the limiting factor in such a series of transitional changes in silage and that no extensive hydrolysis of starch occurred.

Microscopic examination of sections of kernels, leaves, and stems showed no difference in the appearance of the starch granules either with or without stains. No change was discernible in the amount of polarization and in the reaction of the granules with chloral hydrate iodine, enzymes, acids, and alkalies as used by Reichert (12) in his chemical differentiation of the starches.

STARCH.—It would have been desirable to include determinations of starch in the undried and fresh silage. Accurate methods for the starch determination, however, require the sample to be in a fine state of division, and such a condition could not be obtained without consequent deterioration of the silage. It was also found that what actually happens when silage is being dried in a drying oven at 100° C. is not gelatinization and hydrolysis with the acids present, as would ordinarily occur in water mixtures of starch at 100°, but rapid desiccation at a temperature below the gelatinization point of corn starch, which is above 65°. The reason for this is apparent from the fact that the evaporating surface is tremendous and the cooling effect due to vaporization is proportional to the amount of water present. When the free water content approaches zero, then the gelatinization and hydrolytic tendencies of starch also approach zero. The partially dried silage gave no positive tests for soluble starch or erythrodextrin, and the sugar content was not greater than that calculated from the determination of sugar in the juice of the fresh silage.

The Lintner method gave almost identical duplicates even when these were run on different days. The variations in the percentages of starch are within 1.23 per cent and are such that no decrease or synthesis of starch is indicated. The lack of consistency in the variations and their correlation with the other fermentation changes gives further evidence that starch is not changed.

SUMMARY

A study of experimental silage at different stages of fermentation which was normal as regards development of aroma and changes in acidity, alcohol, and sugar content leads to the following conclusions:

(1) Changes in total acidity, alcohol, and sugar are entirely independent of the starch content of the ensiled corn and of the silage produced from it.

(2) The first intermediate products resulting from the decomposition of starch are not present in demonstrable quantities.

(3) The starch content remains constant throughout the fermentation process.

(4) The starch granules remain intact, undergoing no physical change that can be detected by microscopic examination.

(5) Since starch constitutes about 10 per cent of the corn plant at the time of ensiling and represents over 400 calories of available energy per kilogram, the fact that no loss occurs during fermentation is an additional argument in favor of silage as an economical feed.

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EFFECT OF PREMATURE FREEZING ON COMPOSITION OF WHEAT

By M. J. BLISH¹

Assistant Chemist, Montana Agricultural Experiment Station

INTRODUCTION

A consideration of the effects of freezing temperatures upon the chemical composition of the immature wheat kernel is of general interest from a biochemical standpoint and of special interest to those engaged in the study and handling of wheat and its milling products, particularly in the spring-wheat sections. It is of economic importance, especially during the present high prices of wheat and its products, that a large amount of what is popularly called "frosted wheat" is annually classed as fit for nothing better than chicken feed.

This paper presents results of an investigation of the effect of premature freezing on the more important chemical constituents of the wheat kernel, paying special attention to the nitrogen compounds, from which the gluten is formed. Consideration of some of the effects of freezing on the milling and bread-making value of wheat will be taken up in later publications.

Harper (4)² made some analyses of "rusted and frosted" Minnesota wheats in 1889. He reported that the average protein content for rusted and frosted wheats was more than 2 per cent greater than that for the graded wheats, while the ratio of total nitrogen to albuminoid nitrogen was about the same in the damaged and undamaged wheats. The damaged wheat was higher in ash, fat, and fiber but lower in water and carbohydrates than the sound wheat. Different results of analyses of frozen wheat are reported by Foster and Merrill (3, *p. LXVI*) in connection with some Utah samples. Their figures show the total protein to be about 3 per cent lower in frozen than in sound wheat. The frozen wheat samples contained more fiber, fat, and water than the sound wheat. Shutt (5, *p. 117*), in a report of the analyses of Canadian samples of frozen and sound wheats in 1892, found the frozen samples higher in water, fat, fiber, and ash than the sound samples. The percentages of total nitrogen were very nearly the same in both the sound and frozen wheats. In 1907 Shutt (6) determined the albuminoid and nonalbuminoid nitrogen in samples of sound, frosted, and badly frosted wheat

¹ The writer is indebted to Mr. W. F. Day, of the Montana State Grain Laboratory, for milling the wheat samples dealt with in these experiments, and especially to Mr. Edmund Burke, chemist of the Experiment Station, for helpful criticism and advice.

² Reference is made by number (*italic*) to "Literature cited," p. 188.

by the Stutzer method. In badly frosted wheats he found that from 10 to 16 per cent of the nitrogen was in the nonalbuminoid form. There was practically no difference in the relative amounts of these constituents in the flours milled from these wheats, a fact which will be discussed later in this paper.

Analyses for the crude food constituents, conducted along the conventional lines, have brought out several facts. Frozen wheat runs higher in fiber, ash, and crude fat than sound wheat, although the differences are not always great. The carbohydrate content of frozen wheat is lower than that of sound wheat. The total nitrogen content may be higher or lower, depending probably on some other factors. The moisture content varies with storage conditions but is undoubtedly greater in frozen wheat at the normal time of cutting than in sound wheat.

EXPERIMENTAL WORK

In order to obtain samples of sound and frozen wheat of the same varieties and grown as nearly as possible under the same conditions, plots were seeded at intervals of a week, starting at the beginning of the normal seeding period and ending about two months later. This insured the likelihood of securing samples frozen at different stages of growth. Marquis wheat was used in the experiments discussed in this paper. A series of plots was seeded in 1917, beginning May 12 and ending June 30. One 1/40-acre plot was seeded each week during the interval, making a total of eight plots. The same procedure was followed in 1918, starting April 29 and ending June 18. Two series of samples were thus obtained. The soil used was a black sandy loam on the grounds of the Montana Agricultural Experiment Station. All plots were irrigated in the middle of July and obviously were not at the same stage of development when irrigated. In all plots the wheat was cut either shortly after maturity or immediately after the first killing frost.

It will be noted that in each series of samples only the last two plots seeded were badly frozen. In the first series the plot seeded last was severely frozen when in the late milk stage, and in the second series the wheat from the corresponding plot was less severely frozen when in the early dough stage. In the two most severely frozen samples a large percentage of the kernels were green, shrunk, and "blistered." These two samples may be considered to represent very extreme cases, and such instances are likely to occur only under the most exceptional climatic conditions. The plots seeded next to the last ones are probably more typical of conditions which are likely to occur in actual farming practice, the one in the 1917 series being more severely frosted than that in 1918. Although it is difficult to measure the exact degree of frosting or freezing in a given sample of wheat between certain limits, these samples present an appearance quite similar to the majority of frosted wheat samples

which habitually come under the observation of the State Grain Laboratory. The kernels were not shrunken, nor was there more than a small percentage of green kernels. The large majority of kernels from the 1917 series, however, had the well-known blistered appearance extending over the entire surface of the kernel, which is usually conclusive evidence that the grain has been badly frozen before reaching maturity. The wheat from the corresponding plot of the 1918 series was much less blistered than that from the 1917 series. All the other samples presented the appearance of mature wheat and had been cut before the first killing frost. The samples just discussed may be readily identified in the tables which follow.

Special attention is invited to the manner in which the grain from each series was handled after cutting, since there is strong evidence from the chemical analyses that the two different methods of handling and storage exerted a very appreciable effect on the biological activities within the kernel, aside from the effects of freezing. The wheat from the 1917 series was brought to the granary shortly after cutting and thrashed when dry enough to permit. Samples for subsequent analyses were then stored in a room near the college heating plant where the temperature was abnormally high and where the grain soon became drier than grain stored under normal conditions. It remained there for more than a year before being analyzed. The grain from the 1918 series, however, was allowed to remain in the field, after it was cut and shocked, until late in the following January, when it was taken to the granary and later thrashed. This grain was therefore subjected to several months of severe weathering in the field, and after being thrashed a considerable portion of it presented the bleached appearance which is characteristic of grain which has stood in the shock and undergone weathering. In the discussion of the analytical results which follow, attention will be called to chemical differences which have apparently been caused by the different methods of handling the grain from the two series of experimental plots.

EFFECT OF FREEZING ON NITROGEN COMPOUNDS

In studying the chemical composition of the wheat frozen at different stages of growth, particular attention was directed to the effect of freezing on the nitrogen compounds, since it is the gluten-forming proteins of wheat that give flour its bread-making power. The influence of the other constituents of normal wheat flour on its baking strength are for the most part considered to be indirect and are of importance only in so far as they affect the gluten. In order to estimate the extent to which premature freezing arrests the building up of the proteins from the less complex nitrogen compounds, determinations of the amounts of total nitrogen, nonprotein nitrogen, α -amino nitrogen, amid nitrogen, and

ammonia nitrogen were made on the respective samples of sound and frozen wheat, as well as on straight flours milled from the wheats. The extraction of the nonprotein nitrogen and its quantitative separation from the proteins, as well as its concentration to enable the estimation of the various forms in which it exists, was satisfactorily carried out by methods previously published by the writer (2).

Table I shows the distribution of the various forms of nitrogen in the two series of wheat samples described in preceding paragraphs.

In a previous paper (2) it has been shown that while the proteins themselves are completely removed in the method for determining nonprotein nitrogen there still remain some peptids in the solution. Therefore the figures for α -amino nitrogen reported in the tables include the "exposed" α -amino nitrogen of these peptids as well as the α -amino nitrogen of the amino acids. By far the greater part, however, is from the amino acids rather than from the peptids.

It will be noted that the most severely frozen wheat contains two to three times as much total nonprotein nitrogen as the sound wheat. The increase in ammonia and amid nitrogen is proportional to the increase in nonprotein nitrogen, the percentage of these two constituents in terms of the total nonprotein nitrogen remaining practically constant in each series. In the samples of frozen wheat a much larger percentage of the nonprotein nitrogen is in the α -amino form than in the matured samples. In the most severely frozen sample of the 1917 series nine times as much of the total nitrogen of the wheat is in the α -amino form as in the sample which matured earliest.

It is to be noted that the nitrogen in the α -amino and amid forms, as well as total nonprotein nitrogen, runs higher in the mature samples of the 1918 series than in corresponding samples of the 1917 series, while the α -amino nitrogen runs lower in the frozen samples of the 1918 series than in the corresponding samples of the 1917 series. There is much less difference, however, in the figures for total nonprotein nitrogen in the two series, there being nearly the same percentage in the most severely frozen samples of both series. It is strongly suspected that these differences are due to chemical changes caused by allowing the wheat from the 1918 series to stand in the field several months after cutting. Such treatment frequently occurs to Montana wheat in actual farming practice, and its effect on the composition of the kernel will be more thoroughly investigated in the near future, as well as its influence on the baking quality of the flour.

TABLE I.—*Effect of freezing on nitrogen compounds of immature Marquis wheat*

	1917 series, sample No.—				1918 series, sample No.— ^a				
	1.	6.	7.	8.	1300.	1304.	1305.	1306.	1307.
Date seeded.....	May 12.....	June 16.....	June 23.....	June 30.....	Apr. 29.....	May 29.....	June 3.....	June 10.....	June 18.
Date of first killing frost.....	Oct. 17.....	Oct. 17.....	Oct. 17.....	Oct. 17.....	Oct. 8.....	Oct. 8.....	Oct. 8.....	Oct. 8.....	Oct. 8.
Stage of development.....	Mature.....	Mature.....	Immature.....	Late milk stage.	Matured but bleached from lying in field after cutting.	Same as 1300.	Same as 1300.	Slightly immature.	Early dough stage.
Percentage of total nitrogen in wheat.....	2.59	2.67	2.61	2.44	2.59	2.38	2.39	2.17	2.21
Percentage of nonprotein nitrogen in total nitrogen.....	4.17	4.34	7.05	13.98	7.72	5.88	6.09	10.70	13.20
Percentage of a-amino nitrogen in total nitrogen.....	.55	.65	1.84	5.05	1.23	1.00	1.07	1.84	3.25
Percentage of a-amino nitrogen in nonprotein nitrogen.....	13.52	15.18	26.08	36.00	16.00	17.14	17.58	17.24	24.65
Percentage of ammonia nitrogen in nonprotein nitrogen.....	3.45	3.04	3.28	4.20	2.00	2.74	3.62	4.32
Percentage of amid nitrogen in total nitrogen.....	.54	.52	.88	1.51	1.10	.80	.90	1.81	1.92
Percentage of amid nitrogen in nonprotein nitrogen.....	12.96	12.07	12.48	10.82	14.28	14.00	14.96	16.90	14.55

^a The wheat from the plots harvested in the fall of 1918 was left in the field until late January, 1919, when it was thrashed and stored.

Table II shows analyses of straight flours milled from the more important samples referred to in Table I. The samples in Table II are designated by the same numbers as those in Table I, each number followed by the letter "F."

TABLE II.—*Effect of freezing on nitrogen compounds of straight flour from Marquis wheat*

	1917 series, sample No.—			1918 series, sample No.—		
	1 F.	7 F.	8 F.	1300 F.	1306 F.	1307 F.
Percentage of total nitrogen in flour	2.39	2.46	2.31	2.37	2.11	2.11
Percentage of nonprotein nitrogen in total nitrogen	1.84	4.40	10.56	3.05	3.60	5.12
Percentage of α-amino nitrogen in total nitrogen27	1.29	4.85	.57	.53	1.20
Percentage of α-amino nitrogen in nonprotein nitrogen	14.54	29.44	45.90	19.90	14.74	23.33
Percentage of ammonia nitrogen in nonprotein nitrogen	3.20	4.54	2.87	3.40	2.30	2.60
Percentage of amid nitrogen in nonprotein nitrogen	12.73	12.31	10.33	12.64	12.90	10.69
Percentage of amid nitrogen in total nitrogen23	.54	1.09	.38	.46	.55

Table II shows that the percentage of total nonprotein nitrogen is in all cases considerably less in the flour than in the whole wheat, although it is much greater in the frozen sample than in the matured ones, especially in the 1917 series. This is not entirely in agreement with the findings of Shutt (6), who used Stutzer's method and reported that flour milled from frosted wheat contained as high a percentage of its total nitrogen in the albuminoid form as flour from sound wheat, although the frozen whole wheat contained a larger percentage in the nonalbuminoid form than did the sound wheat. His conclusion is that the nonalbuminoid nitrogen compounds are practically all removed by the milling process and may therefore be considered to be located in the bran and germ.

The findings of Shutt agree much more closely with the 1918 series than with the 1917 series. Inspection of the figures for total nonprotein nitrogen in Table II shows that a much greater proportion of the nonprotein nitrogen compounds was removed by milling in the 1918 series than in the 1917 series. This indicates that either the freezing was of such a nature that in one season it affected chiefly the nitrogen compounds in the bran and germ, while in the other it affected the whole kernel, or the difference has been caused by the different methods by which the crops from the two series were handled after cutting, as has previously been discussed in this paper.

EFFECT OF FREEZING ON THE CARBOHYDRATES

A brief study of the effects of premature freezing on the carbohydrates of the wheat kernel was made. To this end wheat samples from both series were analyzed by the methods of Stone (7). The results of these analyses are presented in Table III.

TABLE III.—Some effects of freezing on carbohydrates of Marquis wheat

	1917 series, sample No. —				1918 series, sample No.—			
	1.	6.	7.	9.	1300.	1305.	1306.	1307.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Reducing sugars.....	0.02	0.09	0.30	1.86	0.09	0.11	0.16	2.40
Sucrose.....	1.63	1.35	1.72	1.64	1.65	1.53	1.08	1.27
Dextrin and soluble starch	1.66	1.87	2.10	2.34	1.87	1.75	2.08	2.17

The data in Table III show that the percentage of reducing sugars increases with the severity of the freezing, as would be anticipated. The figures for reducing sugars in the most severely frozen samples of both series offer further evidence that either the freezing in the 1917 series was far more severe than in the 1918 series or the different methods of handling the two series after cutting resulted in different biochemical activities within the kernel. Also, as would be expected, there is more soluble starch and dextrin in the frozen samples than in the matured ones. There seems to be no apparent relationship between the sucrose content and the severity of freezing.

EFFECT OF FREEZING ON ACIDITY

The general effect of freezing on the acidity of the samples of wheat and flour used in these experiments was briefly studied by titrating water extracts with *N*/0.05 alkali, using phenolphthalein, although electrometric titrations with the hydrogen electrode might be preferable. With reference to acidimetric titrations of cereal extracts, Birckner (1) has recently shown that the addition of alcohol to water extracts containing amino compounds increases the acidity of the extracts in proportion to the amount of amino compounds present. Water extracts of the wheat and flour samples in question were therefore titrated with and without alcohol. According to Birckner the difference between the two titrations should be an index to the amino compounds present, and a comparison of these differences with results obtained by the use of Van Slyke's microapparatus (see Tables I and II) should be of interest. In the alcoholic titrations the water extracts were diluted with equal volumes of neutral alcohol. The results are set forth in Table IV.

In examining the values expressed by the differences between the titrations with and without alcohol for the respective samples, it may readily be seen that not only do these values increase as the severity of freezing increases but the extent of the increase in almost all instances keeps pace with the figures for nonprotein and α -amino nitrogen as actually determined and shown in Tables I and II. This is in close agreement with the findings of Birckner (1).

TABLE IV.—*Acidimetric titrations of wheat and flour extracts with and without alcohol*
[50-cc. portions of water extract used, representing 4 gm. of sample]

	1917 series, sample No.—						1918 series, sample No.—					
	1.	7.	8.	1F.	7F.	8F.	1300.	1306.	1307.	1300 F.	1306 F.	1307 F.
Cubic centimeters of <i>N</i> /0.05 sodium hydroxid neutralized without alcohol.....	3.5	4.6	6.8	1.4	2.0	4.1	3.1	3.8	4.0	1.5	1.1	1.7
Cubic centimeters of <i>N</i> /0.05 sodium hydroxid neutralized with alcohol.....	6.0	8.4	14.4	2.2	4.4	10.0	6.1	7.0	8.8	3.0	2.5	4.0
Difference due to amino compounds.....	2.5	3.8	7.6	0.8	2.4	5.9	3.0	3.2	4.8	1.5	1.4	2.3
Percentage of nonprotein nitrogen in total nitrogen ^a	4.17	7.05	13.98	1.84	4.40	10.56	7.72	10.70	13.20	3.05	3.60	5.12
Percentage of a-amino nitrogen in nonprotein nitrogen ^a	13.52	26.08	36.06	14.54	29.44	45.90	16.00	17.24	24.65	19.90	14.74	23.33

^a Figures taken from Tables I and II.

SUMMARY

(1) Premature freezing affects the chemical composition of wheat and the flour milled therefrom.

(2) Frozen wheat contains larger amounts of nonprotein nitrogen, reducing sugars, and acid-reacting constituents than does sound wheat.

(3) The nonprotein nitrogen of frozen wheat carries a considerably higher percentage of a-amino nitrogen than that of sound wheat.

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BEHAVIOR OF THE CITRUS-CANKER ORGANISM IN THE SOIL¹

By H. ATHERTON LEE

*Pathologist, Fruit—Disease Investigations, Bureau of Plant Industry,
United States Department of Agriculture*

INTRODUCTION

It is a commonly accepted idea among fruit growers and horticulturists that the citrus-canker organism, *Pseudomonas citri* Hasse, lives and multiplies in the soil. There has been considerable field evidence to support this view. Frequently after an infected tree has been cut or burned down, young shoots have come up from the roots and have been found to be cankered. Thus Wolf² writes—

That it [*P. citri*] remains alive in the soil is indicated by the appearance of diseased sprouts from the roots of diseased trees which are burned.

Stevens³ in 1915 reported the successful cultivation of *P. citri* in sterilized soil, and this has been accepted by a number of horticulturists as sufficient evidence to conclude that the canker organism is a soil inhabitant.

The presence or absence of the canker organism in the soil is an important question, and the use of many of the eradication and quarantine methods depends upon a knowledge of the behavior of the canker organism in the soil. The question resolves itself into three points: (1) whether

¹The investigations reported in this paper were carried on at the Lamaso Agricultural Experiment Station of the Philippine Bureau of Agriculture. The writer is greatly indebted to Col. Adrian Hernandez, Director, and Mr. S. Apostol, Chief, Plant Industry Division of the Philippine Bureau of Agriculture, for the facilities afforded at Lamaso and for much other assistance. Thanks are also due Mr. Francisco Galang, Superintendent of the Station at Lamaso, for helpfulness at all times.

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²WOLF, Frederick A. CITRUS CANKER. In Jour. Agr. Research, v. 6, no. 2, p. 69-100, 8 fig., pl. 9-11. 1916. Literature cited, p. 98-99.

³STEVENS, H. E. CITRUS CANKER-III. Fla. Agr. Exp. Sta. Bul. 128, 20 p., 6 fig. 1915.

P. citri is able to live actively—that is, increase and multiply within the soil; (2) whether it exists simply passively and does not increase and multiply; or (3) whether it is killed within the soil.

The problem has been attacked previously by the writer and other investigators by attempting to plate out soil samples and thus show the presence of the canker organism in the soil. These attempts have usually given negative results. However, such negative results have been inconclusive because of the large number of the soil organisms which would appear in the plates, making it difficult to identify *P. citri* even if it were present. Investigations were therefore undertaken at Lamao, P. I., with the purpose of attacking this question with different experimental methods.

EXPERIMENT I

Fifty-five culture tubes of orchard soil from Lamao were prepared. These were autoclaved twice, one hour each time, at 45 pounds pressure, 24 hours intervening between periods of steaming. Fifty-five tubes of the same soil were prepared but were not sterilized. Each tube of sterilized soil was inoculated with 2 cc. of a heavy infusion of *P. citri* in sterile water, precautions being used as far as possible to avoid contamination. The tubes of unsterilized soil were inoculated each with 2 cc. of the same infusion, all processes being identical except that one series of tubes was sterilized while the other was not.

Five of the sterilized tubes and 5 of the unsterilized tubes were taken; portions were removed from each with a spatula (a separate spatula being used for each tube); and infusions were then made from each of these 10 portions. These infusions were made in dry sterilized test tubes, but tap water was used for the liquid medium. Inoculations from each infusion were made upon the upper and lower surfaces of five young, actively growing pummelo¹ leaves, *Citrus maxima* (syn. *C. grandis*, *C. decumana*). Forty punctures were made in each leaf. The leaves were then bound in waxed paper with wet cotton to maintain a moist atmosphere. The whole was covered with opaque paper to prevent burning by the sun.

This procedure was repeated each day for a period of 15 days, a new series of five tubes of sterilized soil and a new series of tubes of unsterilized soil being used each day. The inoculation data and results are given in Tables I and II. The percentages expressed are based upon the number of positive takes resulting from the total of 200 punctures on 5 leaves. Where stomatal infections occur they are counted as wound infections.

¹ Following the usage of W. T. Swingle in Bailey's Standard Cyclopedia of Horticulture, the term pummelo is used in its usual East Indian sense to include varieties of *Citrus grandis* distinct from the grapefruit group of the West Indies and the United States.

TABLE I.—*Inoculations on young pummelo leaves from infusions of untreated soil in tubes made on consecutive days after inoculation with P. citri*

Leaves No.	Infusion tube No.	Number of days after inoculation of tubes.	Date of inoculation from tubes to leaves.	Infections from 200 punctures.	Date of observation.
1 to 5	1	2	Oct. 23, 1918	93 per cent positive...	Nov. 2, 1918.
6 to 10	2	2do.....	36 per cent positive...	Do.
11 to 15	3	2do.....do.....	Do.
16 to 20	4	2do.....	22 per cent positive...	Do.
21 to 25	5	2do.....	61½ per cent positive.	Do.
26 to 30	6	3	Oct. 24, 1918	51½ per cent positive.	Nov. 4, 1918.
31 to 35	7	3do.....	6 per cent positive...	Do.
36 to 40	8	3do.....	12 per cent positive...	Do.
41 to 45	9	3do.....	4½ per cent positive.	Do.
46 to 50	10	3do.....	All negative.....	Do.
51 to 55	11	4	Oct. 25, 1918	½ of 1 per cent positive.	Do.
56 to 60	12	4do.....	All negative.....	Do.
61 to 65	13	4do.....	3 per cent positive...	Do.
66 to 70	14	4do.....	½ of 1 per cent positive.	Do.
71 to 75	15	4do.....	3 per cent positive...	Do.
76 to 80	16	5	Oct. 26, 1918	All negative.....	Nov. 5, 1918.
81 to 85	17	5do.....	½ of 1 per cent positive.	Do.
86 to 90	18	5do.....	All negative.....	Do.
91 to 95	19	5do.....	½ of 1 per cent positive.	Do.
96 to 100	20	5do.....	1 per cent positive...	Do.
101 to 105	21	7	Oct. 28, 1918	All negative.....	Nov. 9, 1918.
106 to 110	22	7do.....do.....	Do.
111 to 115	23	7do.....do.....	Do.
116 to 120	24	7do.....do.....	Do.
121 to 125	25	7do.....do.....	Do.
126 to 130	26	9	Oct. 30, 1918do.....	Nov. 20, 1918.
131 to 135	27	9do.....do.....	Do.
136 to 140	28	9do.....do.....	Do.
141 to 145	29	9do.....	9½ per cent positive.	Do.
146 to 150	30	9do.....	All negative.....	Do.
151 to 155	31	11	Nov. 1, 1918do.....	Do.
156 to 160	32	11do.....do.....	Do.
161 to 165	33	11do.....do.....	Do.
166 to 170	34	11do.....do.....	Do.
171 to 175	35	11do.....do.....	Do.
176 to 180	36	14	Nov. 4, 1918do.....	Do.
181 to 185	37	14do.....do.....	Do.
186 to 190	38	14do.....do.....	Do.
191 to 195	39	14do.....do.....	Do.
196 to 200	40	14do.....do.....	Do.
201 to 205	41	14do.....do.....	Do.
206 to 210	42	14do.....do.....	Do.
211 to 215	43	14do.....do.....	Do.
216 to 220	44	14do.....do.....	Do.
221 to 225	45	14do.....do.....	Do.
226 to 230	46	14do.....do.....	Do.
231 to 235	47	14do.....do.....	Do.
236 to 240	48	14do.....do.....	Do.
241 to 245	49	14do.....do.....	Do.
246 to 250	50	14do.....do.....	Do.

TABLE II.—*Inoculations on young pummelo leaves from infusions of autoclaved soil in tubes made on consecutive days after inoculation with P. citri*

Leaves No.	Infusion tube No.	Number of days after inoculation of tubes.	Date of inoculation from tubes to leaves.	Infections from 200 punctures.	Date of observation.
1 to 5	1	2	Oct. 23, 1918	100 per cent positive..	Nov. 2, 1918.
6 to 10	2	2do.....	99½ per cent positive..	Do.
11 to 15	3	2do.....	100 per cent positive..	Do.
16 to 20	4	2do.....do.....	Do.
21 to 25	5	2do.....do.....	Do.
26 to 30	6	3	Oct. 24, 1918do.....	Nov. 4, 1918.
31 to 35	7	3do.....do.....	Do.
36 to 40	8	3do.....do.....	Do.
41 to 45	9	3do.....do.....	Do.
46 to 50	10	3do.....do.....	Do.
51 to 55	11	4	Oct. 25, 1918do.....	Do.
56 to 60	12	4do.....do.....	Do.
61 to 65	13	4do.....do.....	Do.
66 to 70	14	4do.....do.....	Do.
71 to 75	15	4do.....do.....	Do.
76 to 80	16	5	Oct. 26, 1918do.....	Nov. 5, 1918.
81 to 85	17	5do.....do.....	Do.
86 to 90	18	5do.....do.....	Do.
91 to 95	19	5do.....do.....	Do.
96 to 100	20	5do.....do.....	Do.
101 to 105	21	7	Oct. 28, 1918do.....	Nov. 9, 1918.
106 to 110	22	7do.....do.....	Do.
111 to 115	23	7do.....do.....	Do.
116 to 120	24	7do.....do.....	Do.
121 to 125	25	7do.....do.....	Do..
126 to 130	26	9	Oct. 30, 1918do.....	Nov. 20, 1918.
131 to 135	27	9do.....do.....	Do.
136 to 140	28	9do.....do.....	Do.
141 to 145	29	9do.....do.....	Do.
146 to 150	30	9do.....do.....	Do.
151 to 155	31	11	Nov. 1, 1918do.....	Do.
156 to 160	32	11do.....do.....	Do.
161 to 165	33	11do.....do.....	Do.
166 to 170	34	11do.....do.....	Do.
171 to 175	35	11do.....do.....	Do.
176 to 180	36	14	Nov. 4, 1918do.....	Do.
181 to 185	37	14do.....do.....	Do.
186 to 190	38	14do.....do.....	Do.
191 to 195	39	14do.....	92½ per cent positive..	Do.
196 to 200	40	14do.....	100 per cent positive..	Do.

SUMMARY OF EXPERIMENT I

Inoculations made upon young pummelo leaves from infusions made from autoclaved soil tubes inoculated with *P. citri* were uniformly 100 per cent positive or nearly so for 14 days following the inoculation of the soil tubes with *P. citri*. Inoculations from infusions from tubes of unsterilized soil in which *P. citri* was inoculated gave uniformly positive results on young pummelo trees during the first 3 days. Thereafter the percentages of positive results were low upon the fourth, fifth, and seventh days. On the ninth day there were but a few positive results

from a total of 1,000 punctures, and on the eleventh and fourteenth days 4,000 puncture inoculations from the infusions were entirely negative. The evidence of this experiment therefore points to a gradual dying out of the canker organism in unsterilized soil, although in the sterilized soil the canker bacteria are very active.

EXPERIMENT II

This experiment was undertaken to obtain all possible information upon the condition of *P. citri* in orchard soils.

Rain had fallen intermittently every day for 15 days. The Ellen grapefruit tree selected for this experiment showed 100 per cent of the leaves cankered, and in many cases the leaves had over 50 cankers apiece—that is to say, the tree was badly affected with canker and a drop of water could hardly fall to the ground from this tree without having been in contact with cankers.

During a violent shower, rain dripping from the leaves of this tree was collected in five culture tubes. These tubes were then carried to the isolation plots where citrus-canker had been excluded. From each tube of the rain water five young, actively growing grapefruit leaves were inoculated on upper and lower surfaces, each leaf being punctured at the same time with 40 needle stabs. The heavily cankered grapefruit tree was then cut down and removed, and all fallen leaves were removed from the ground. Soil from beneath the tree was then placed in five culture tubes, infusions were made and taken to the isolation plots, and five leaves were inoculated from each infusion. Forty punctures were made on each leaf, and both upper and lower surfaces were coated.

The twigs bearing the leaves inoculated with the infusion as well as those inoculated from the drip water were wrapped in paraffin paper with a piece of moistened cotton. The paraffin paper was then covered with opaque paper. A muslin tent was spread over the soil about the stump of the Ellen grapefruit tree after all fallen leaves had been removed. The tent prevented infected leaves from being blown upon the soil but allowed active play of rain and air as under normal conditions. The percentages of infection are given in Tables III and IV.

TABLE III.—Inoculations on young pummelo leaves from rain water collected from the leaves of a badly cankered grapefruit tree

Leaves No.	Infusion tube No.	Date of inoculation on leaves.	Infections from 200 punctures.	Date of observation.
1 to 5	1	July 20, 1918	3 per cent positive.....	July 31, 1918.
6 to 10	2do.....	All negative.....	Do.
11 to 15	3do.....	4½ per cent positive.....	Do.
16 to 20	4do.....	19½ per cent positive.....	Do.
21 to 25	5do.....	36½ per cent positive.....	Do.

TABLE IV.—*Inoculations on young pummelo leaves made immediately after rain and on consecutive days following the rain from infusions of orchard soil from beneath a heavily infected grapefruit tree*

Leaves No.	Infusion tube No.	Number of days between rain and inoculation.	Date of inoculation on leaves.	Infections from 200 punctures.	Date of observation.
1 to 5	1	Immediately after rain.	July 20, 1918	All negative....	July 31, 1918.
6 to 10	2do.....do.....	8½ per cent positive.	Do.
11 to 15	3do.....do.....	4½ per cent positive.	Do.
16 to 20	4do.....do.....	12½ per cent positive.	Do.
21 to 25	5do.....do.....	1 per cent positive.	Do.
26 to 30	6	1	July 21, 1918	All negative....	Do.
31 to 35	7	1do.....	½ of 1 per cent positive.	Do.
36 to 40	8	1do.....	All negative....	Do.
41 to 45	9	1do.....do.....	Do.
46 to 50	10	1do.....do.....	Do.
51 to 55	11	2	July 22, 1918	½ of 1 per cent positive.	Do.
56 to 60	12	2do.....	All negative....	Do.
61 to 65	13	2do.....do.....	Do.
66 to 70	14	2do.....do.....	Do.
71 to 75	15	2do.....do.....	Do.
76 to 80	16	3	July 23, 1918do.....	Aug. 2, 1918.
81 to 85	17	3do.....do.....	Do.
86 to 90	18	3do.....do.....	Do.
91 to 95	19	3do.....do.....	Do.
96 to 100	20	3do.....do.....	Do.
101 to 105	21	4	July 24, 1918do.....	Do.
106 to 110	22	4do.....do.....	Do.
111 to 115	23	4do.....do.....	Do.
116 to 120	24	4do.....do.....	Do.
121 to 125	25	4do.....do.....	Do.
126 to 130	26	5	July 25, 1918do.....	Aug. 3, 1918.
131 to 135	27	5do.....do.....	Do.
136 to 140	28	5do.....do.....	Do.
141 to 145	29	5do.....do.....	Do.
146 to 150	30	5do.....do.....	Do.
151 to 155	31	7	July 27, 1918do.....	Aug. 16, 1918.
156 to 160	32	7do.....do.....	Do.
161 to 165	33	7do.....do.....	Do.
166 to 170	34	7do.....do.....	Do.
171 to 175	35	7do.....do.....	Do.
176 to 180	36	9	July 29, 1918do.....	Do.
181 to 185	37	9do.....do.....	Do.
186 to 190	38	9do.....do.....	Do.
191 to 195	39	9do.....do.....	Do.
196 to 200	40	9do.....do.....	Do.

SUMMARY OF EXPERIMENT II

Inoculations made from rain water collected from the foliage of a heavily infected grapefruit tree gave positive results upon young pummelo leaves. Inoculations made from infusions of the soil beneath such a heavily infected tree also gave positive results on pummelo leaves immediately following the rain. On the first day after the rain there

was one positive result and on the second day following the rain there was a positive result. Thereafter on the third, fourth, fifth, seventh, and ninth days the results were entirely negative. On these days a total of 125 leaves, or 5,000 punctures were inoculated with the soil infusion, and all remained negative.

The conclusion is reached, then, that although the canker organism was present immediately following the rain, in this case the citrus-canker organism has died out in the orchard soil.

REPETITION OF EXPERIMENTS I AND II

The field data have been very extensive in support of the theory that the canker bacteria can exist and multiply in the soil. Since the idea has been so firmly held by growers and horticulturists that the canker organism does live in the soil, and because the data presented in the two preceding experiments indicate the contrary to be the case, both these experiments were repeated.

Experiment I was repeated, and the original results were entirely corroborated. It was found that *P. citri* was abundant in the unsterilized soil tubes during the first, second, and third days; during the fourth, fifth, seventh, and ninth days the inoculations were but very slightly positive; on the fourteenth day the organism showed three positive results from a total of 4,000 punctures. In the sterilized soil tubes *P. citri* gave almost uniformly 100 per cent results up to and including the fourteenth day.

Experiment II was carried through three times. The first trial has been reported here in detail. For the second and third trials the same methods were used. In a second trial the water dripping from the foliage of an infected grapefruit tree was shown to contain *P. citri* in a large percentage of cases. The soil beneath the tree, immediately following the rain, also gave a large number of positive results. On the second day after the rain and thereafter for four days inoculations from the soil beneath the same tree gave entirely negative results on the pummelo leaves. In a third trial no tests were made with the rain water on the leaves, but immediately following the rain a large number of positive results were obtained on pummelo leaves from inoculations with the soil infusion from beneath the cankered foliage. On the first day after the rain a few positive results were obtained from the soil infusions, but on the second day none of the inoculations resulted positively. On the third and eighth days there were again a few positive results, but on the tenth day 2,000 inoculations made from the soil upon the pummelo leaves remained entirely negative. These second and third trials entirely corroborate the experiments reported above and indicate that the citrus canker organism is entirely killed in orchard soils.

The tests of orchard soil were carried on at different seasons of the year and are representative of the conditions in the soil in very different

climatic periods in the Philippines. Two of the series of inoculations with orchard soil infusions were carried on in the middle of the rainy season when the soil was kept constantly wet by the rains. The third series of inoculations was carried on at the beginning of the dry season when the soil dried out and became dusty to a considerable extent. The attempt was made to secure the soil for each day's infusion at different depths. Soil was frequently taken from the surface and just as frequently from a depth of 10 inches. It is thought that the inoculations shown here were made from soil infusions which are entirely representative of the different conditions in the Lamao soils.

Inasmuch as the question of the existence or nonexistence of *P. citri* in the soil is an important point in canker control work, the following test was undertaken to corroborate further the preceding experiments.

EXPERIMENT III

INOCULATED SOIL, IN BOXES

Orchard soil was autoclaved twice, one hour each time at 45 pounds pressure. The soil was placed in thin layers on plates, so that the steam would penetrate easily. The autoclaved soil was placed in a seed-house flat which measured 18 by 24 by 5 inches. The soil was air-dried and was inoculated with 1,500 cc. of an infusion of *P. citri* in sterile water. This flat was then placed at a level with the soil and covered with cheesecloth to prevent animals from disturbing it. The flat received the full play of sun, wind, and rain and was exposed to the same conditions as exist beneath a tree in the orchard.

Another flat of the same size containing unsterilized soil was inoculated with an equal amount of an identical infusion. This flat was placed under identical conditions with the flat of sterilized soil but at several yards' distance to prevent distribution of the canker organism too easily; it was also covered with cheesecloth.

On the first day after inoculation, a small portion of the inoculated soil from the autoclaved flat was removed with a spatula to a clean dry-sterilized culture tube. To this about 10 cc. of tap water were added; the tube was shaken vigorously for several minutes; and the resulting infusion was spread upon the upper and lower surfaces of five actively growing pummelo leaves. Each leaf was then punctured 40 times with a new needle, and a new coating of the infusion was spread over the leaves and over the punctures. For this spreading of the infusion small cotton swabs such as are used for collecting diphtheria cocci from suspected cases were used. A new swab was used for each tube of infusion.

Five infusions were made each successive day from the flat of inoculated autoclaved soil. On each successive day five infusions were made in the same way from the unsterilized inoculated soil, and each of these was spread upon five actively growing leaves, each leaf being subsequently punctured 40 times.

Thus inoculations were made each day from 10 infusions of soil. These 10 infusions were identical in every way except that 5 were made from a flat of soil which had been inoculated with *P. citri* after being sterilized while the other 5 were made from a flat of soil which had been inoculated without being sterilized. Tables V and VI give the results of the inoculations.

TABLE V.—*Inoculation of young pummelo leaves from infusions of untreated soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions*

Leaves No.	Infusion tube No.	Number of days after inoculation into soil.	Date of inoculation on leaves.	Infections from 200 punctures.	Date of observation.
1 to 5	1	1	Oct. 23, 1918	77 per cent positive..	Nov. 2, 1918.
6 to 10	2	1do.....	80 per cent positive...	Do.
11 to 15	3	1do.....	87 per cent positive ..	Do.
16 to 20	4	1do.....	100 per cent positive.	Do.
21 to 25	5	1do.....	53½ per cent positive.	Do.
26 to 30	6	2	Oct. 24, 1918	22 per cent positive..	Nov. 4, 1918.
31 to 35	7	2do.....	28 per cent positive..	Do.
36 to 40	8	2do.....	54½ per cent positive.	Do.
41 to 45	9	2do.....	78½ per cent positive.	Do.
46 to 50	10	2do.....	73 per cent positive..	Do.
51 to 55	11	3	Oct. 25, 1918	16½ per cent positive.	Do.
56 to 60	12	3do.....	8½ per cent positive..	Do.
61 to 65	13	3do.....	18½ per cent positive.	Do.
66 to 70	14	3do.....	15½ per cent positive.	Do.
71 to 75	15	3do.....	21 per cent positive..	Do.
76 to 80	16	4	Oct. 26, 1918	All negative.....	Nov. 5, 1918.
81 to 85	17	4do.....do.....	Do.
86 to 90	18	4do.....	1½ per cent positive.	Do.
91 to 95	19	4do.....	All negative.....	Do.
96 to 100	20	4do.....	6 per cent positive ...	Do.
101 to 105	21	6	Oct. 28, 1918	All negative.....	Nov. 9, 1918.
106 to 110	22	6do.....do.....	Do.
111 to 115	23	6do.....do.....	Do.
116 to 120	24	6do.....do.....	Do.
121 to 125	25	6do.....do.....	Do.
126 to 130	26	8	Oct. 30, 1918do.....	Nov. 20, 1918.
131 to 135	27	8do.....do.....	Do.
136 to 140	28	8do.....do.....	Do.
141 to 145	29	8do.....do.....	Do.
146 to 150	30	8do.....do.....	Do.
151 to 155	31	10	Nov. 1, 1918do.....	Do.
156 to 160	32	10do.....do.....	Do.
161 to 165	33	10do.....do.....	Do.
166 to 170	34	10do.....do.....	Do.
171 to 175	35	10do.....do.....	Do.
176 to 180 ^a	36	12do.....do.....do.....
181 to 185	37	12	Nov. 3, 1918	All negative.....	Nov. 20, 1918.
186 to 190	38	12do.....do.....	Do.
191 to 195	39	12do.....do.....	Do.
196 to 200	40	12do.....do.....	Do.

^a Leaves 176 to 180 were inoculated and then found to be already naturally infected at insect injuries. These leaves were therefore cut off Nov. 3, 1918, and were not carried in the experiment.

TABLE V.—*Inoculation of young pummelo leaves from infusions of untreated soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions—Continued*

Leaves No.	Infusion tube No.	Number of days after inoculation into soil.	Date of inoculation on leaves.	Infections from 200 punctures.	Date of observation.
201 to 205	41	14	Nov. 5, 1918	All negative.....	Nov. 20, 1918.
206 to 210	42	14do.....do.....	Do.
211 to 215	43	14do.....do.....	Do.
216 to 220	44	14do.....do.....	Do.
221 to 225	45	14do.....do.....	Do.
226 to 230	46	14do.....do.....	Do.
231 to 235	47	14do.....do.....	Do.
236 to 240	48	14do.....do.....	Do.
241 to 245	49	14do.....do.....	Do.
246 to 250	50	14do.....do.....	Do.
251 to 255	51	14do.....do.....	Do.
256 to 260	52	14do.....do.....	Do.
261 to 265	53	14do.....do.....	Do.
266 to 270	54	14do.....do.....	Do.
271 to 275	55	14do.....do.....	Do.

TABLE VI.—*Inoculations on young pummelo leaves from infusions of autoclaved soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions*

Leaves No.	Infusion tube No.	Number of days after inoculation into soil.	Date of inoculation on leaves.	Infections from 200 punctures.	Date of observation.
1 to 5	1	1	Oct. 23, 1918	96 per cent positive ..	Nov. 2, 1918.
6 to 10	2	1do.....do.....	Do.
11 to 15	3	1do.....	98 per cent positive ..	Do.
16 to 20	4	1do.....	95½ per cent positive.	Do.
21 to 25	5	1do.....	99 per cent positive ..	Do.
26 to 30	6	2	Oct. 24, 1918	100 per cent positive .	Oct. 30, 1918.
31 to 35	7	2do.....do.....	Do.
36 to 40	8	2do.....do.....	Do.
41 to 45	9	2do.....do.....	Nov. 4, 1918.
46 to 50	10	2do.....do.....	Do.
51 to 55	11	3	Oct. 25, 1918do.....	Do.
56 to 60	12	3do.....do.....	Do.
61 to 65	13	3do.....	99½ per cent positive.	Do.
66 to 70	14	3do.....	100 per cent positive..	Do.
71 to 75	15	3do.....do.....	Do.
76 to 80	16	4	Oct. 26, 1918do.....	Nov. 5, 1918.
81 to 85	17	4do.....	96 per cent positive ..	Do.
86 to 90	18	4do.....	100 per cent positive .	Do.
91 to 95	19	4do.....do.....	Do.
96 to 100	20	4do.....do.....	Do.
101 to 105	21	6	Oct. 28, 1918	95½ per cent positive.	Nov. 9, 1918.
106 to 110	22	6do.....	100 per cent positive .	Do.
111 to 115	23	6do.....	99 per cent positive..	Do.
116 to 120	24	6do.....	100 per cent positive.	Do.
121 to 125	25	6do.....	94½ per cent positive.	Do.

TABLE VI.—Inoculations on young pummelo leaves from infusions of autoclaved soil in a box made on consecutive days after inoculation with *P. citri*. The inoculated soil was placed in the orchard to simulate field conditions—Continued

Leaves No.	Infusion tube No.	Number of days after inoculation into soil.	Date of inoculation on leaves.	Infections from 200 punctures.	Date of observation.
126 to 130	26	8	Oct. 30, 1918	100 per cent positive .	Nov. 9, 1918.
131 to 135	27	8do.....do.....	Do.
136 to 140	28	8do.....do.....	Do.
141 to 145	29	8do.....	76 per cent positive..	Do.
146 to 150	30	8do.....	100 per cent positive.	Do.
151 to 155	31	10	Nov. 1, 1918do.....	Nov. 20, 1918.
156 to 160	32	10do.....do.....	Do.
161 to 165	33	10do.....do.....	Do.
166 to 170	34	10do.....	99½ per cent positive.	Do.
171 to 175	35	10do.....	100 per cent positive .	Do.
176 to 180	36	12	Nov. 3, 1918do.....	Do.
181 to 185	37	12do.....	83½ per cent positive.	Do.
186 to 190	38	12do.....	100 per cent positive .	Do.
191 to 195	39	12do.....	93½ per cent positive.	Do.
196 to 200	40	12do.....	96 per cent positive ..	Do.
201 to 205	41	14	Nov. 5, 1918	100 per cent positive..	Do.
206 to 210	42	14do.....do.....	Do.
211 to 215	43	14do.....do.....	Do.
216 to 220	44	14do.....do.....	Do.
221 to 225	45	14do.....do.....	Do.

SUMMARY OF EXPERIMENT III

It will be seen that inoculations made from the untreated soil were highly positive on the first day following inoculation with *P. citri*. On the second day there was a slight diminution of the positive results, and on the third day the percentages of positive results were very much lower. On the fourth day the larger part of the inoculations were entirely negative. On the sixth, eighth, tenth, twelfth, and fourteenth days following inoculation, all inoculations were entirely negative. That is, six days after the inoculation with a heavy infusion of *P. citri* in untreated soil, 170 leaves were inoculated, each with 40 punctures, or a total of 6,800 punctures; all remained negative. At the same time inoculations made on consecutive days following inoculations of autoclaved soil with *P. citri* were highly positive the first day, increased to almost uniformly 100 per cent positive results on the second day, and continued at 100 per cent for 14 days.

The full significance of this may perhaps be grasped more readily by a brief recapitulation. A dense infusion of virulent active canker organisms was heavily inoculated into a box of soil entirely untreated and but recently removed from the orchard. This box of inoculated but otherwise untreated soil was kept under orchard conditions during the experiment.

Six days after the inoculation with the heavy infusion no indications of the organism could be obtained from this soil. As a control upon the conditions a similar box of soil, alike in every detail except that it had been autoclaved, was inoculated; it showed the continuance of the canker bacteria throughout 14 days, and the bacteria were apparently as numerous on the fourteenth day as on the first.

These experimental results, as well as those with the tubed soils, indicate that the canker organism does not increase and multiply or live even a passive existence in the normal soil but is quickly killed out. Inasmuch, however, as it will live in soil from which all other organisms are excluded, there is indication that in unsterilized soil the activities of the normal soil organisms are antagonistic to the existence of *P. citri*.

The following results obtained by a different experimental procedure still further corroborate the previous conclusions.

EXPERIMENT IV

This experiment was conducted to show the persistence or absence of the canker bacteria by growing susceptible plants in inoculated soils.

Ten bamboo pots were autoclaved and subsequently filled with soil twice autoclaved. These soil pots were then heavily inoculated with a dense infusion of *P. citri* in sterile water. On the same day 30 bamboo pots filled with unsterilized soil were inoculated with the canker organism from similar infusions.

Pots 1 to 5 of sterilized, inoculated soil were immediately planted each with 10 seeds from *Citrus trifoliata* fruits; pots 11 to 20 of unsterilized, inoculated soil were also immediately planted each with 10 seeds of *C. trifoliata*. After an interval of 5 days 10 more pots of unsterilized, inoculated soil were planted each with 10 seeds; and after an interval of 10 days 10 pots of unsterilized soil and 5 more pots of sterilized soil were planted, each pot with 10 *C. trifoliata* seeds.

It was the intention, of course, that the *Citrus trifoliata* seedlings resulting would be very susceptible and in growing through the inoculated soil would become infected if the canker organism still remained alive within the soil.

Running parallel with these series of inoculated soil pots, a series of orchard soil pots was operated as follows: Ten pots were filled with soil taken from beneath a heavily infected grapefruit tree, immediately following a rain, and each pot was planted with *Citrus trifoliata* seeds. The tree was cut down and all sources of infection were removed from the soil; then 10 days later 10 more pots were filled with the same soil and similarly planted.

All pots, those containing orchard soil naturally infected and those artificially inoculated, were covered with cheesecloth after planting to prevent the ingress and egress of insects which might spread infection.

The two series of pots were kept separated in the dense tropical woods at Lamao. The inoculation and planting data with results are given in Tables VII and VIII.

TABLE VII.—*Results of sprouting seeds and growing young plants of Citrus trifoliata in pots of soil artificially inoculated with canker bacteria*

Pot No.	Treatment of soil.	Date of inoculation.	Date of planting.	Results.	Date of examination.
1	Sterilized ..	Oct. 22, 1918	Oct. 22, 1918	No trees.	Jan. 16, 1919.
2 to 5do.....do.....do.....	9 trees, no infections.	Do.
6 to 10do.....do.....	Nov. 1, 1918	10 trees, no infections.	Do.
11 to 20	Unsterilizeddo.....	Oct. 22, 1918	31 trees, no infections.	Do.
31 to 33do.....do.....	Oct. 27, 1918	7 trees, no infections.	Do.
34do.....do.....do.....	No trees.	Do.
35 to 40do.....do.....do.....	19 trees, no infections.	Do.
51 to 58do.....do.....	Nov. 1, 1918do.....	Do.
59 to 61do.....do.....do.....	No trees.	Do.

TABLE VIII.—*Results of sprouting seeds and growing young plants of Citrus trifoliata in pots of soil naturally infected with canker bacteria in the orchard*

Pot No.	Condition of soil.	Length of time after rain.	Date of planting.	Results.	Date of examination.
21 to 22	Naturally infected.	Immediately after rain.	Oct. 23, 1918	No trees.	Jan. 16, 1919.
23 to 30do.....do.....do.....	15 trees, no infections.	Do.
41 to 42do.....	5 days.	Oct. 28, 1918	No trees.	Do.
43 to 50do.....do.....do.....	18 trees, no infections.	Do.
62 to 66do.....	13 days.	Nov. 5, 1918	No trees.	Do.
67 to 69do.....do.....do.....	4 trees, no infections.	Do.
70do.....do.....do.....	No trees.	Do.
71do.....do.....do.....	1 tree, no infections.	Do.

SUMMARY OF EXPERIMENT IV

One hundred and thirty-three seedling *Citrus trifoliata* trees were sprouted in soil pots. These pots had been inoculated with the canker organism, either by artificial or natural means, from 35 to 40 days previous to the sprouting of the seeds. None of the seedlings at any time showed canker, although they were kept for 45 days after they appeared above the ground. The seedlings were from seed taken from heavily infected *C. trifoliata* fruits on badly infected *C. trifoliata* trees; there can be no doubt as to the general susceptibility of the stock. The strain of the organism used in inoculating the soil was the same as that which produced lesions

upon the lansones (*Lansium domesticum*), and there can be no question as to its virulence. The temperatures and humidity were at all times favorable for the development of canker.

Theoretically, criticism of the results of this experiment might be raised, since none of the trees, even the controls in sterilized inoculated soil, showed canker. Practically, however, there is a very good explanation. The seeds did not begin to sprout and the young shoots to push through the soil until the first week in December—that is, 35 days after soil was inoculated. During this time the sterilized soil pots were exposed in the Lamao woods, protected only from contamination by coarse cheesecloth. Under these conditions it could be expected that a few weeks after being placed in the woods the soil in the pots would be well inoculated with the ordinary soil flora and the canker organism would then be killed out. Another explanation might be that the normal young seedlings of *Citrus trifoliata* are possibly resistant to citrus canker infection, in which event the value of this method of testing for soil infection would be lessened.

SUMMARY OF RESULTS OF EXPERIMENTS

It has been shown in two separate experiments that *P. citri* lives and may even increase in culture tubes of sterilized soil throughout a period of 14 days or more. On the other hand, tubes of identical soil, handled in an identical manner with the exception of not being autoclaved, showed the canker organism to be entirely killed out within a period of 6 days.

In three similar experiments, representing two distinct seasonal periods, it was shown that the canker organism can be found in the soil beneath a heavily infected tree on the day immediately following the rain and on the second and third days following. Thereafter there is no indication of the canker organism in the soil.

In another experiment a box of soil was autoclaved and then inoculated with *P. citri*. This box, placed in the orchard to simulate field conditions, showed no decrease in the activity of the canker organism during a period of 14 days after inoculation. A box of similar soil, treated in an identical manner with the exception of not being autoclaved, showed the canker organism to be entirely killed out within a period of 6 days.

In the last experiment seeds were planted in nonsterile soil which had been inoculated with a heavy infusion of *P. citri*. The seeds which germinated and pushed through the soil 40 days after inoculation never showed any sign of canker although they were kept for 45 days after their appearance above the soil.

The results of each series of experiments point to the dying out of the canker organism in untreated soils. The indication is that the normal soil organisms are antagonistic in some way to the existence of *P. citri* in the soil.

The soil at Lamao is a sandy loam and seems to be of alluvial origin. There is little or no indication of decaying organic matter in the soil

and there is no reason to base a supposition for unusual bacterial activity on such grounds. The soil used in the experiments was taken from directly beneath trees of the Ellen grapefruit variety and was plowed, cultivated, and hoed according to usual orchard practices. The treatment of the soil differed very little from that usually practiced in the United States.

APPLICATION OF RESULTS

The writer would prefer that any applications of these findings be made by the field men, who are in the best position to judge the merits of different methods in eradication work. The following suggestion might be made, however, from a theoretical viewpoint.

It is frequently stated that canker is carried from orchard to orchard upon muddy feet or in the earth upon farm implements. These statements appear to be based upon a wrong conception of the character of the canker organism, and it would seem probable that the disease bacteria are carried upon dry portions of clothing and implements rather than in the earth. These experiments should therefore serve not to decrease the vigilance of quarantine measures but to increase the precautions to eliminate all sources for reinfection and dissemination of canker; for inasmuch as these experiments indicate that the canker organism does not live in the soil, field data which seem to indicate that *P. citri* is a soil inhabitant must be explained as indications of a source of reinfection overlooked or of a careless transfer of the organisms by farm animals or man.

SOME POSSIBLE SOURCES FOR REINFECTION BY THE CANKER ORGANISM

It has been demonstrated by Peltier and Neal¹ that the canker organism may overwinter in the bark tissue of citrus trees. The following observations may supplement their findings as to the means of overwintering or survival.

In the Philippine Islands lesions very much resembling those of citrus-canker were observed upon the mature wood of grapefruit trees (*Citrus maxima*) and lime (*C. aurantifolia*). These lesions were of a slightly lighter brown color than the normal bark and consisted of eruptions of tissue very similar to cankers upon leaves. Examinations of frozen sections of such eruptions revealed the typical structure of citrus-canker and the masses of bacteria distributed as in leaf cankers. *P. citri* was subsequently isolated from these lesions. Photographs (Pl. 36) show these mature wood cankers better than a description. The mature wood cankers were also observed upon navel orange trees (*C. sinensis*) in orchards in Japan.

Close examination has revealed that these mature wood cankers are by no means uncommon on lime, grapefruit, and sweet orange trees;

¹ PELTIER, George L., and NEAL, David C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. In Jour. Agr. Research, v. 14, no. 11, p. 523-524, pl. 58. 1918.

and their manner of occurrence indicates that wounds are not necessary for infection. They are commonly to be found upon branches as large as 2 or even 3 inches in diameter, the wood of which has entirely hardened and matured. One case has been observed of such cankers on the trunk of a lime tree 6 inches in diameter. Such cankers have never been seen on species other than the lime, the sweet orange, and the grapefruit. Cankers occurring in this way do not cause the killing of the limbs or branches, their seriousness consisting chiefly in affording constant sources for reinfection of foliage and fruit. Such cankers are also easily overlooked, inasmuch as they are small and of the same color as the normal bark.

The presence of such cankers suggested that cankers might also occur upon the roots of trees. Inoculations were therefore attempted upon roots with *P. citri* by means of needle punctures. The inoculations reacted slowly, but in 30 days examination showed that some of the punctures were undoubtedly positive. Control punctures with tap water were negative. The inoculations were then made repeatedly. The best series of results is selected here for presentation in Table IX. A photograph (Pl. 37, A) also shows some of these results. Mature trees, actively growing and thrifty, were selected for inoculation.

TABLE IX.—Results of inoculations with *P. citri* by means of needle punctures into roots of *Citrus sinensis*

Inoculation No.	Inoculum.	Diameter of root.	Date of inoculation.	Result.	Date of examination.
		<i>Mm.</i>			
119	<i>P. citri</i> culture.....	4	Dec. 5, 1917..	Positive.....	Feb. 15, 1918.
120do.....	4do.....do.....	Do.
121do.....	4do.....do.....	Do.
122do.....	4do.....do.....	Do.
123do.....	4do.....do.....	Do.
124do.....	4do.....do.....	Do.
125do.....	4do.....do.....	Do.
126do.....	4do.....do.....	Do.
127do.....	4do.....do.....	Do.
128do.....	4do.....do.....	Do.
129	Tap water.....	4do.....	Negative.....	Do.
130do.....	4do.....do.....	Do.
131do.....	4do.....do.....	Do.
132do.....	4do.....do.....	Do.
133do.....	4do.....do.....	Do.
134do.....	8do.....do.....	Do.
135do.....	8do.....	Swelling (no eruption).	Do.
136do.....	8do.....	Lost.....	Do.
137do.....	8do.....	Negative.....	Do.
138do.....	8do.....do.....	Do.

The inoculations were made with a needle, and the punctures were covered with moist cotton and wrapped in paraffin paper, then in opaque paper, and covered with earth.

From such positive results of inoculations *P. citri* was several times reisolated; and such cultures reinoculated on leaves of *Citrus maxima* gave positive results. There is therefore a possibility, considered however to be small, that the canker organism is carried on the roots.

In digging in the soil beneath citrus trees in the Philippines, leaves have been uncovered upon which cankers were found. These leaves were skeletonized by the soil organisms, the lignified tissues apparently resisting the action of the soil organisms while the cellulose parts of the leaf blade had entirely disappeared. Canker lesions upon such buried leaves also seem to resist the dissolving action of the soil bacteria. Photographs (Pl. 37, B) show the persistence of cankers upon such buried skeletonized leaves. Whether cankered leaves which have been buried and subsequently uncovered may possibly furnish another means of carrying the canker organism over in spite of control measures is a question that deserves special experimental investigation.

In Florida there have been many cases of seemingly thorough eradication of the disease followed by a new outbreak, even after considerable periods of inactivity. Such outbreaks at the time have been the cause for considerable conjecture and speculation. It is possible that the results presented here may point to hitherto overlooked sources of new infection occurring after a period of latency.

SUMMARY

(1) Experimental evidence is given to show that *P. citri* disappears from unsterilized soil in tubes and boxes usually within six days after they are inoculated. *P. citri* inoculated in sterilized soil increases and multiplies. Since the main difference in this latter case is the exclusion of the normal soil organisms, the disappearance of *P. citri* seems to be ascribable to the antagonistic effect of such soil inhabitants.

(2) In soil under orchard conditions, the canker organism is found to disappear even more rapidly than in the soil confined in boxes or culture tubes.

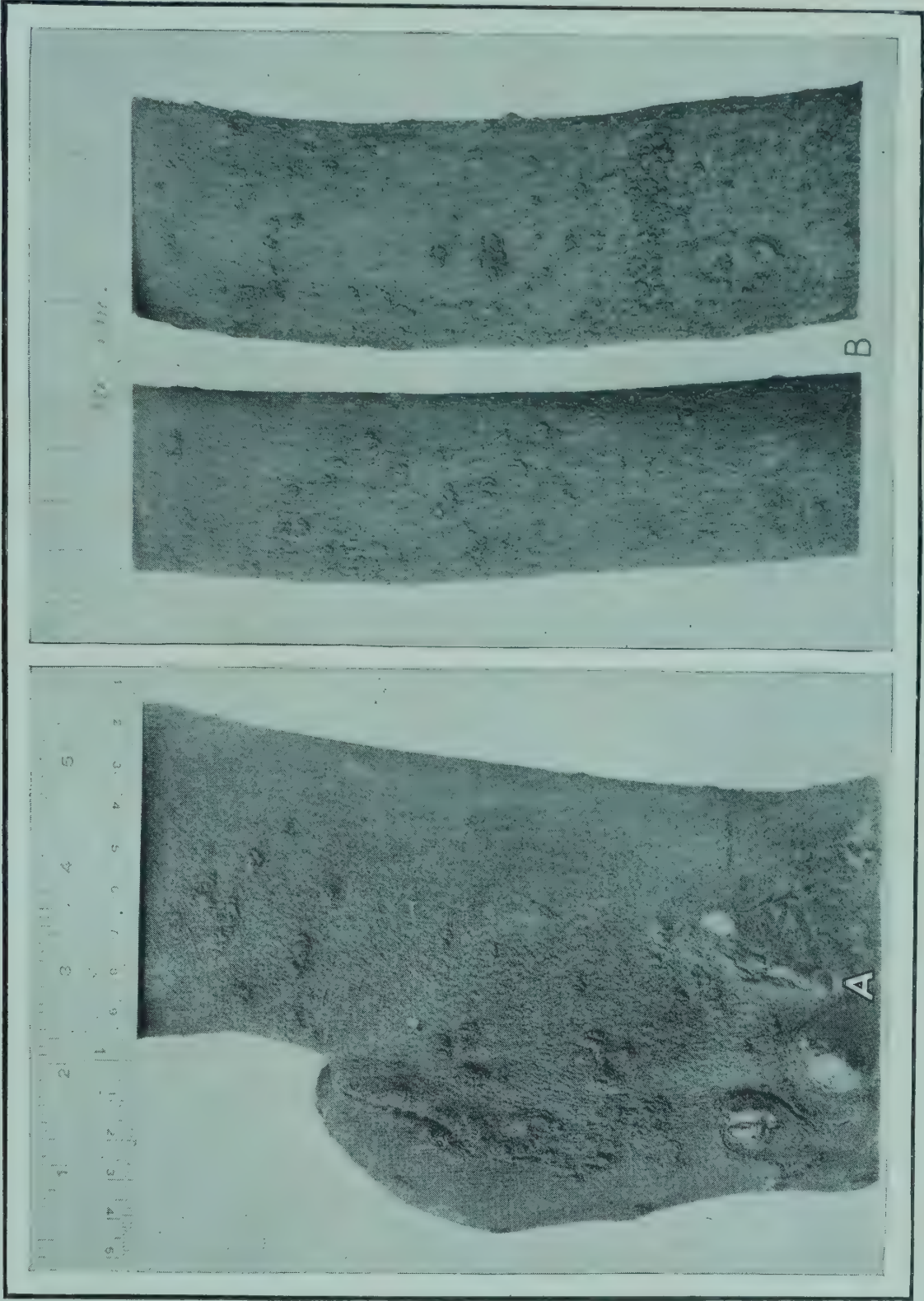
(3) Seeds were planted in pots of soil naturally infected with the canker organism and in pots of soil artificially inoculated. The seedlings came through the soil and developed normally without any canker, thus corroborating the conclusion that the canker bacteria are killed out in normal soils.

(4) Cankers upon mature wood of citrus trees and positive inoculations upon the roots of citrus trees are shown. Cankers upon buried leaves and mature wood and roots as possible sources of holding over the canker organism are suggested.

PLATE 36

A.—Citrus-cankers on mature wood of trunk of *Citrus aurantifolia*. Slightly reduced.

B.—Citrus-cankers on mature wood of branches of *Citrus aurantifolia*. Natural size.



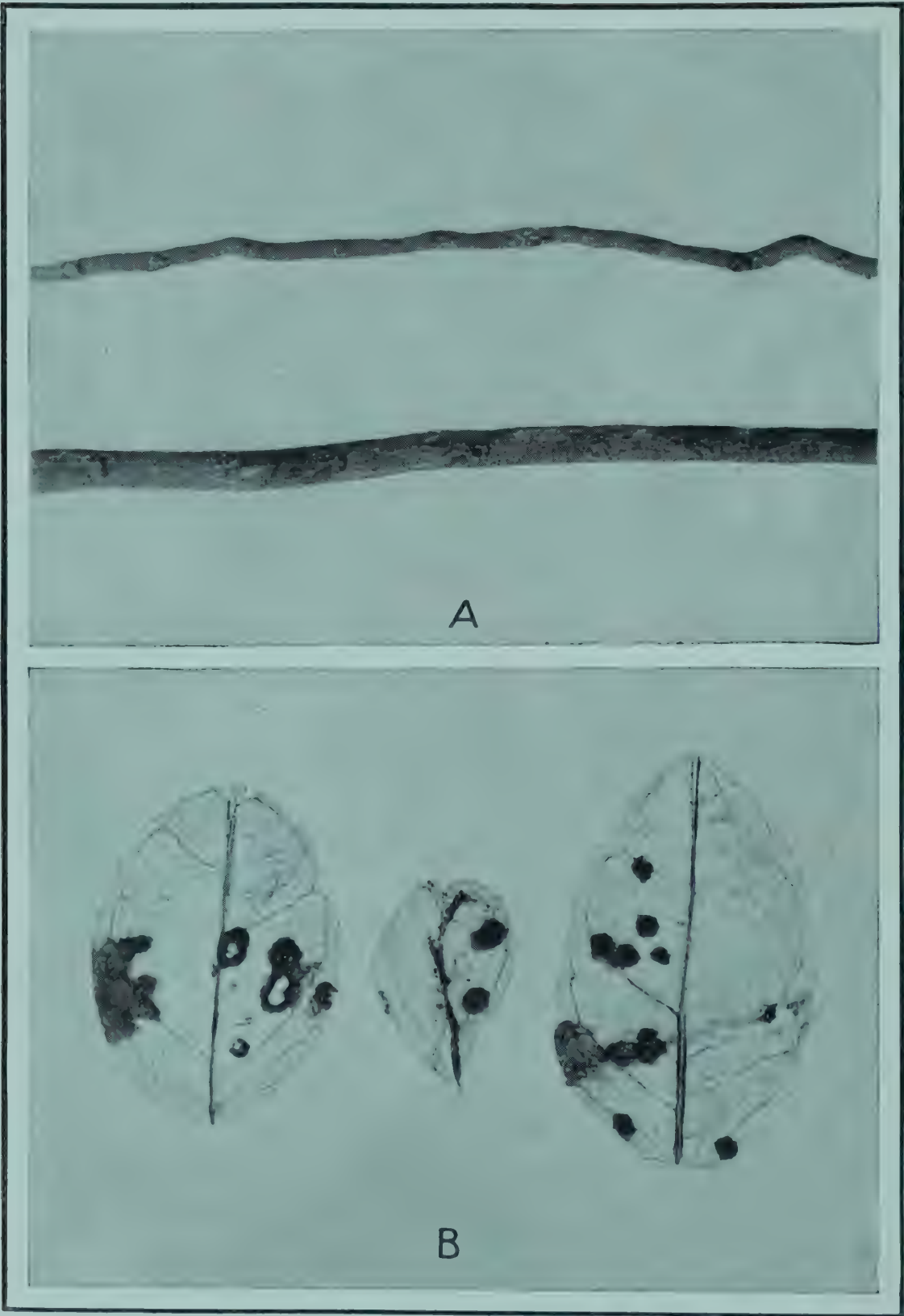


PLATE 37

A.—Results of inoculations with *Pseudomonas citri* upon roots of sweet orange (*Citrus sinensis*). Natural size.

B.—Skeletonized leaves of Ellen grapefruit recovered from buried soil. The leaf blade is entirely decomposed, leaving only the lignified veins and the cankered tissue. Natural size.

DECLINE OF PSEUDOMONAS CITRI IN THE SOIL

By H. R. FULTON

*Pathologist, Fruit-Disease Investigations, Bureau of Plant Industry, United States
Department of Agriculture*

This investigation was undertaken primarily to determine whether or not the citrus-canker organism, *Pseudomonas citri* Hasse, is capable of persisting in the soil to such an extent as to make the soil an important medium in holding over or disseminating the organism.

EXPERIMENTAL METHODS

The work has been conducted in an isolated greenhouse near Washington, D. C. During the tests the soils were kept in ordinary 4- or 6-inch earthenware flower pots in duplicate, triplicate, or quadruplicate sets for each test. For the original inoculation of the soil it was found most satisfactory to use washings from potato cylinder cultures 2 to 10 days old. One such culture tube diluted with 200 cc. of water would give heavy inoculation in a 4-inch pot. The bacterial suspension was well mixed with the upper 3 inches of the soil, and samples were taken from this portion from at least three different points.

Because of the preponderance of more rapidly growing soil organisms, ordinary plating methods are inadequate for determining the abundance of *P. citri* in soil samples, and recourse was had to inoculation of punctured mature grapefruit leaves with graded dilutions of washings from the soil to be tested. The procedure was as follows: A sample of about 20 gm. of the soil was removed with a sterile spoon to a sterile Petri dish, and enough sterile distilled water was added to give an excess of about 10 cc. beyond saturation. This was well stirred, and 1 cc. of the soil solution was transferred to another Petri dish in which 9 cc. of sterile distilled water had been previously placed. The first washing described above is referred to as the 1/1 dilution in this paper, and the second as the 1/10 dilution. In a similar way dilutions of 1/100, 1/1,000 or beyond were made from the original soil wash water. Small wefts of sterile absorbent cotton were placed in each dish, one for each leaf to be inoculated. The grapefruit seedlings used were grown in 2, 2½, or 3 inch pots. They averaged 6 or 8 inches in height and had as a rule 8 to 12 leaves. Usually 5 leaves per plant were used for inoculation, and each leaf was punctured at 100 points. A simple device for making these punctures rapidly and accurately was improvised by inserting 10 sewing needles through a small cork stopper. This "punch" was readily sterilized by flaming the needles, was convenient to handle, made the punctures in a uniform group pattern, and thus contributed materially to the rapidity

and accuracy of the work. A leaf was inoculated by wiping both under and upper surface of the freshly punctured portion with a cotton swab from a dilution dish, the swab being finally left on the upper surface. The inoculated plant was wrapped in paraffin paper, which served to retain moisture and to prevent accidental contamination from outside sources. Other plants were inoculated with pure cultures of *P. citri* as controls, and others were set up with the swabs merely wet with sterile water. The series were held at least a week in glass inoculation cases where conditions were near the optimum for canker development; later they were removed to the greenhouse benches. The first observations and records were made as a rule two to four weeks after inoculation. Final records were deferred until four to eight weeks after inoculation in order to insure the detection of any unusually slow development of infection such as occurred when the inoculum contained only a few organisms. The records show that between 90 and 95 per cent of the infections were apparent at the first observation and that no material increase was secured by holding beyond the second observation.

Variations of this method were tried out during its evolutionary development and to some extent in routine work as special considerations seemed to warrant. In many of the earlier series absorbent cotton wicks from small bottles of sterile water were placed in contact with the inoculation swabs on the leaves. This precaution to secure a prolonged moist condition proved to be unnecessary. An inoculum of mud paste, made by adding only a little water to the soil sample and applied with a backing of cloth or cotton as a sort of poultice over the punctured area, gave distinctly fewer infections than the soil solution in much greater dilutions. In cases where a large quantity of liquid inoculum could be prepared, a very effective method of inoculation was to dip the whole top of the test plant with its punctured leaves, keeping it submerged for an hour or longer, with several shakings during the period. In a few instances the test plants were so placed that the punctured leaves remained buried in the soil of the pots for a day or two. Tests were made of placing the plants under an air exhaust after soil water had been applied to their leaf surfaces. The soil solution was centrifuged to concentrate the canker organisms when they were very few, but this was without definitely satisfactory results. Still another method¹ employed was to atomize the leaves with sterile water, sift over them the rather dry soil to be tested, and keep the leaf surfaces moist for several days by holding the plants in a moist chamber and by repeatedly atomizing them with sterile water.

It was not apparent that any of the modifications of testing procedure could be relied upon to give a larger percentage of infections than the standard method, or to show the presence of *P. citri* when the standard method failed to give positive results.

¹ This method was first used by Miss Clara H. Hasse, of this office.

SENSITIVENESS OF DILUTION METHOD OF TESTING

In various tests involving several thousand plants, the standard testing method, which employs graded dilutions of the soil washing for inoculation on punctured grapefruit leaves, has proved reasonably sensitive in detecting the presence of viable *P. citri* in the soil. It is satisfactory for securing a rather definite idea of the relative numbers of this organism at the various times of sampling.

To test the efficiency of the method, dilutions in decimal series were made from a loopful of potato cylinder culture of *P. citri* distributed in the requisite number of cubic centimeters of sterile distilled water and were carried well beyond the vanishing point. One-cc. portions from each dilution were plated in beef agar for *P. citri* counts. Cotton swabs were dipped in the remainder of each dilution and applied to grapefruit leaves having 100 punctures each. Measurement showed these swabs to carry an average of 0.7 cc. of the liquid. The results of two independent tests are given in Table I.

TABLE I.—Comparison between number of infections on grapefruit leaves and counts on poured plates, using graded dilutions of *P. citri*

TEST A

	$1/10,000$	$1/100,000$	$1/1,000,000$	$1/10,000,000$	$1/100,000,000$	$1/1,000,000,000$	$1/10,000,000,000$
Average number of infections, 20 leaves tested.....	76	14	2	0.2	0	0	0
Average count for 1 cc. inoculum, 5 plates.....	22,300	2,500	300	32	1	0	0
Average number of organisms applied per leaf.....	15,600	1,750	210	22
Average number of organisms per infection.....	205	125	105	110

TEST B

	$1/10,000$	$1/100,000$	$1/1,000,000$	$1/10,000,000$	$1/100,000,000$	$1/1,000,000,000$	$1/10,000,000,000$
Average number of infections, 16 leaves tested.....	69	9	1.4	0.13	0	0	0
Average count for 1 cc. inoculum, 6 plates.....	25,000	2,500	277	28	2.3	0.5	0.2
Average number of organisms applied per leaf.....	17,500	1,750	194	20
Average number of organisms per infection.....	253	194	139	154

The method apparently gives evidence of something like 30 organisms per cubic centimeter of inoculum, provided as many as 20 test leaves with 100 punctures each are used. The upper limit of sensitiveness would evidently be reached when numbers of bacteria are sufficient to infect practically all punctures, and diminution of sensitiveness would appear earlier. The ratio between infections per leaf and bacteria

applied is seen to be fairly uniform for the more critical lower ranges in both tests and lies between 1 to 100 and 1 to 200.

On the mature grapefruit leaves used for the tests there was practically no infection except at the freshly made punctures, and the counts are therefore free from errors that might have arisen from secondary spread if the unwounded tissue had been highly susceptible.

Two things are involved in the causation of infection by very dilute inoculum: (1) the chance for the organisms to reach the punctures and (2) the average number of organisms required to initiate infection successfully at a given point. In using cotton swabs, a considerable proportion of the organisms would be held at a distance from the leaf surface, and of those actually in the surface moisture film many would be at relatively great distances from punctures. On the other hand, motility of the organism and the possibility of rapid numerical increase by division would increase the chance for infection. As to the minimum number of *P. citri* organisms necessary to set up infection on reaching a given puncture, further careful experiments must be conducted before an opinion can be ventured.

PERSISTENCE IN VARIOUS TYPES OF SOIL

To secure as great diversity as possible with types of soil conveniently at hand the following kinds were selected: (1) stiff clay subsoil thrown out from an excavation several months previously; (2) leaf mold screened from ground surface in forest; (3) rotting compost of sod and manure thoroughly decayed; (4) garden soil, a clay loam of moderate fertility. Four-inch pots were used in duplicate for each soil type. The inoculum for each pot amounted to 10 cc. of a 2-day beef bouillon culture of *P. citri* mixed with about one-fifth of the washing from a 6-day potato cylinder culture, the whole being diluted to 100 cc. and evenly mixed with the upper 3 inches of soil in the pot. Inoculations were made on August 14, 1918. The pots were held in the greenhouse shaded from direct sunlight, and were given ordinary watering. Each percentage in Table II is based on the number of infections developed in 17 days in a total of 600 leaf punctures and represents the average of duplicate pots of each soil type.

TABLE II.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of solutions from four types of soil made at various intervals after the soil had been inoculated with *P. citri*

Number of days between inoculation and sampling.	Clay subsoil.				Leaf mold.				Compost.				Garden soil.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	28	52	15	0.3	55	63	13	2	35	16	8.3	3	60	33	32	2.3
2.....	9.2	11	0.8	0	17	8.7	0.8	0	11	22	57	4.7	60	33	28	14
5.....	.3	0	0	0	0.5	.2	0	0	38	32	5.2	.7	57	38	6.7	.5
9.....	0	0	0	0	.2	0	0	0	8.0	0.2	.2	0	3.5	0.7	0	.2
14.....	0	0	0	0	0	0	0	0

P. citri evidently decreased very rapidly in all these soils and apparently reached the vanishing point in all in less than 14 days. The rate of decrease was most rapid for the clay subsoil, slightly less so for the leaf mold, and distinctly slower for the compost and garden soil.

A second test was begun September 7, 1918, with new lots of soil from the same sources with the addition of well-washed sand from a creek bed, and a mixture of equal parts of the leaf mold and garden soil used in the earlier experiment. The initial inoculation was about 50 per cent heavier than in the preceding series. In order of rapidity of decrease clay subsoil proved again to be first, followed by leaf mold, sand, compost, garden soil, and mixture of leaf mold and compost. At the termination of this test, 14 days after inoculation, the red clay was the only one giving negative results; and the percentages for the leaf mold, compost, and garden soil were approximately those given for the ninth day in Table I. This longer persistence in the second test may reasonably be attributed to the higher initial inoculation of the soil.

In other experiments the following citrus soils from Florida were used: (1) from Orlando, intermediate between high and low pine soil types, unusually rich in humus; (2) similar to (1) but naturally poor; (3) similar to (2) but from a poorly drained spot; (4) from Bradentown, low pine land of low fertility; (5) from Bradentown, typical muck, extremely rich in humus; (6) from Winter Park, high hammock type; (7) from Winter Park, low hammock type. The samples, as a rule, reached the laboratory and were set up before becoming dry. The usual dilutions to 1/1,000 were run, but for brevity the percentages from the 1/1 dilution only are given in Table III. At the higher dilutions the commencement of decline was evident at the second sampling for all types, whereas this decline is not evident from the 1/1 figures of the table until the fifth or sixth day. The tests were made during September and October, 1918, in three distinct series, as indicated in the table. The second and third were conducted by Miss Clara H. Hasse, of this office, through whose courtesy the results have been furnished for this publication. The percentages are based on infection development from 600 punctures.

TABLE III.—Percentages of infection on grapefruit leaves inoculated with 1/1 soil solution at various intervals after the soil had been inoculated with *P. citri*

Series 1.				Series 2.			Series 3.		
Number of days between inoculation and sampling.	Soil 1.	Soil 2.	Soil 3.	Number of days between inoculation and sampling.	Soil 4.	Soil 5.	Number of days between inoculation and sampling.	Soil 6.	Soil 7.
0.....	88	93	80	0.....	31	62	0.....	92	83
2.....	90	93	88	3.....	61	98
5.....	45	50	33	6.....	48	60	6.....	34	41
9.....	6.3	5.3	0.7	10.....	2.2	2.8	10.....	6.8	4.5
14.....	2.5	0	.5	16.....	.3	3.7	15.....	4.8	1.5
.....	48.....	1.0	7.1	22.....	.2	.5
.....	56.....	0	4.2	52.....	.2	0

There is a marked decline preceding the tenth day in all of the soils of Table III. But scattering infections are apparent over a much longer period, and no one of these soils could be safely declared free of *P. citri* at the times of discontinuance of the respective tests. It is a fact that regular watering of the pots was overlooked during the latter part of the longer tests, and the dry condition probably contributed to the long persistence. Special evidence on this point is given later in this paper.

Florida soils were also used in a number of other special tests, accounts of which follow throughout this paper.

Samples of soil from citrus plantings at Biloxi and Big Point, Miss., were artificially inoculated and tested at 6-day intervals for persistence of *P. citri*. The results were negative on the twelfth day and afterwards.

INFLUENCE OF DEGREE OF INITIAL SOIL INOCULATION

A series was set up September 16, 1918, using three degrees of inoculum, one five times and another one-fifth the usual medium degree. Unfortunately this series was discontinued on the twelfth day, just when the decline from the heavy inoculation was beginning to be pronounced. A second test was begun October 20, 1919. Greenhouse potting soil was used. The medium inoculation consisted of 0.4 of the washings from a potato cylinder culture for each of the duplicate pots. The heavy inoculation was 10 times this, and the light inoculation one-tenth. The pots were kept in the greenhouse, were shaded, and were given ordinary watering. Each percentage given in Table IV is based on 2,000 inoculated punctures.

TABLE IV.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solution at various intervals after the soil had been inoculated with *P. citri* in different degrees

Number of days between inoculation and sampling.	Heavy inoculation.				Medium inoculation.				Light inoculation.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	62	23	5.8	0	9.8	4.8	0.3	0.6	6.0	0	0	0
2.....	33	30	5.5	1.0	9.1	3.0	.4	.2	1.0	0.1	0	0.05
4.....	11	3.5	1.6	.4	1.4	0	.05	0	0	.05	0	0
7.....	4.6	1.0	.4	0	0	0	0	0	0	0	0	0
9.....	.3	0	0	0	0	0	0	0	0	0	0	0
11.....	.05	0	0	0	0	0	0	0	0	0	0	0
14.....	.3	.05	0	0	0	0	0	0	0	0	0	0
18.....	0	0	0	0	0	0	0	0	0	0
23.....	.2	0	0	0	0	0	0
30.....	0	0	0

It is not understood why all the initial soil inoculations in this series turned out to be so far below the expected degree. What was intended for heavy soil inoculation ran considerably below that ordinarily used in other experi-

mental tests. At the same time it is probably as high as would be encountered in citrus plantings under infected trees; and the whole series may be regarded as representing high, medium, and low degrees of soil infection under natural conditions.

The differences are apparently not so much in rate of decline as in time required to reach the zero level from the different initial levels of inoculation.

INFLUENCE OF SOIL TEMPERATURE ON PERSISTENCE

The test for low temperature effect, series 1, which is reported in Table V, was made by exposing the inoculated potting soil to outdoor temperatures, beginning October 11, 1918. During the test the minimum daily readings ranged from 60° to 23° F., and the maximum daily readings from 83 to 58°. For moderate temperatures, exposure was made in the greenhouse, with daily means averaging about 15° higher than outside. Series 2 was begun October 20, 1919, using an incubator at 95° for the high range and the greenhouse for the moderate. The actual soil temperatures 2 inches below the surface were taken, the high temperature test ranging from 86° to 90° and the moderate from 68° to 72°. Percentages for series 1 are based on 600 inoculated punctures, and for series 2 on 2,000.

TABLE V.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solutions at various intervals after the soil had been inoculated with *P. citri* and had been held at different temperatures

Series 1.								Series 2.									
Days between inoculation and sampling.	Moderate temperature.				Low temperature.				Days between inoculation and sampling.	Moderate temperature.				High temperature.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000		1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....			66	7.2	53	12	0....	9.8	4.8	0.3	0.6	5.8	1.4	1.3	0.3
3.....		97	62	9.5	...	68	60	42	2....	9.1	3.0	.4	.2	0.1	0	0	0
7.....		3.3	3.3	1.8	73	50	4....	1.4	0	.05	0	.3	0	0	0
10....	24	2.0	.3	1.7	45	90	37	14	7....	0	0	0	0	0	0	0	0
15....	0.3	.3	0	...	24	90	25	...	9....	0	0	0	0	0	0	0	0
19....			a1.3		a21	...	11....	0	0	0	0	0	0	0	0
28....			a.2		a3.1	...	14....	0	0	0	0	0	0	0	0
36....			a0		a0	...	18....	0	0	0	0	0	0	0	0
42....			a0		a0	...	23....	0	0	0	0	0	0	0	0

a Inoculated by dipping plant top in liquid.

There is a very evident retardation of the rate of decline at the lower temperatures. A second series of October 23, 1918, confirms this for a still lower range of temperature. The higher temperatures seem to accelerate the decline, but the unfortunate low initial inoculation of the soil requires a repetition of the test. At the time of handling series 1, the influence of soil dryness in prolonging persistence had not been determined,

and too little attention was given to watering the pots regularly during the latter part of the experiment. But the outside pots retained moisture much better than those inside, and any difference would have been against longer persistence in them.

INFLUENCE OF SOIL MOISTURE ON PERSISTENCE

A test was begun September 2, 1918, using ordinary potting soil. Inoculation was with a mixture of beef bouillon and potato cylinder cultures. One set of duplicate pots was kept near the saturation point by watering thoroughly every other day. A second lot was restored at each watering to the halfway point between saturation and the original air-dry condition of the soil. The third set was left unwatered. The percentages in Table VI are based on 600 inoculated punctures.

TABLE VI.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solution at various intervals after the soil had been inoculated with *P. citri* and had been held at three moisture contents

Number of days between inoculation and sampling.	Soil continuously saturated.				Soil moderately watered.				Soil air dry.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	62	70	77	68	68	55	80	47	43	80	85	57
1.....	54	23	38	32	57	38	18	43	55	83	47	42
3.....	53	72	37	8.7	67	60	45	21	67	78	71	70
5.....	48	55	23	11	75	48	43	4.3	65	53	7.2	7
7.....	2.2	0.8	0.2	0	10	4.2	1	0	11	3.3	1.2	0
12.....	1.5	.7	.3	0	0.3	.2	0	0	1.8	4.5	.8	0
17.....	0	.32	0	3.5	.2

The foregoing test shows no very pronounced or definite differences in rate of decrease. The slight differences tend toward lag with decrease of moisture, the moderately watered soil showing possibly less rapid decline than the saturated, and the air-dry soil showing still greater retardation.

Further tests of moderately wet soil as compared with dry were made at different times with three lots of Florida soil and are reported in Table VII. The soil for series 1 was from a "sand-soak" spot at Estero, Fla.; for series 2, from high pine land near Leesburg, Fla.; and for series 3, from intermediate pine land at Orlando, Fla. These tests were made by Miss Clara H. Hasse, of this office, during October and November, 1918, and through her courtesy are presented here. Only the 1/1 dilutions are included in Table VII, since in each series the results from higher dilutions were in accord with these. The percentages are based on inoculation of 600 punctures.

The first series indicates distinctly a retarded decline and prolonged persistence in the dry soil. The second series, with another type, shows no decided difference between the wet and dry. In the third series the initial decline was more rapid in the dry than in the wet soil.

TABLE VII.—Percentages of infection on grapefruit leaves inoculated with 1/1 solutions of three Florida soils at various intervals after the soils had been inoculated with *P. citri* and had been held at two moisture contents

Number of days between inoculation and sampling.	Series 1.		Series 2.		Series 3.	
	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.
0.....	98	98	100	97	95	100
3.....	98	100	100	95	86	19
6.....	83	74	77	90	8.8	0.5
9.....	82	94	90	64	5.7	.7
14 or 15 ^a	9.7	93	63	23	0	.3
21.....	6.3	40	1.8	1.7
44 or 43.....	0	18	0	0
50 to 54.....	0	0	0	0	0	0

^a Where two numbers appear for days they indicate slight differences in the sampling periods for the several series.

In Table VIII two series, one set up June 12 and one July 8, 1919, are compared. Both were with soil from Orlando, Fla., of the same type but collected at different times. The inoculum for each 6-inch pot in the two series was from four potato cylinder cultures. The first series was kept well watered. The second was dried overnight after the original inoculation and kept air-dry thereafter. The percentages for the first series are averaged for four similar pots and are based on 4,000 inoculated punctures; those for the second are for three pots and are based on 3,000 inoculated punctures. These series were set up and conducted for approximately the first two months by Miss Clara H. Hasse.

TABLE VIII.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solution at various intervals after the soil had been inoculated with *P. citri* and had been held at two moisture contents

Number of days between inoculation and sampling.	Series 1, moist soil.				Series 2, dry soil.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	59	73	70	60	94	97	98	73
2.....	66	93	87	92	22	7.5	2.4	0.4
4.....	40	17	50	13	11	2.0	2.9	.1
7, 6 ^a	44	44	11	3.9	2.6	2.1	.4	0
9, 8.....	17	14	2.5	1.6	3.9	2.1	.5	0
11, 10.....	13	8.3	3.0	1.0	23	4.6	.8	0
13.....	2.1	2.4	.6	.02	30	4.0	.9	.3
18.....	2.8	1.2	.3	.2	16	2.7	.4	12
21, 23.....	.6	.4	.03	0	32	9.6	.9	.3
33, 34.....	0	.03	0	20	1.7	.1	0
40, 43.....	.03	0	0	3.7	1.1	.3
52, 54.....	0	.03	06	.2	.03
60, 62.....	0	0	0	1.7	.5
69, 71.....	0	0	1.5	.6
78, 80.....	0	3.0	.9
88, 90.....	04
97, 99.....	01
106, 108.....	01
116, 118.....	005
125, 133.....	01
..., 166.....1

^a Where two numbers appear for days the first applies to series 1 and the second to series 2.

Heavy initial inoculation, frequent samplings over a long period, and inoculation at each sampling of 40 or 30 grapefruit leaves with 100 punctures each for the respective series render the results in these series especially noteworthy. In the moist series, after the fourth day, one notes a general equality of percentages on diagonals extending downward and to the left from any of the 1/1,000 figures. For example, the 1/1,000 dilution on the fourth day, the 1/100 on the seventh, the 1/10 on the ninth, and the 1/1 on the eleventh are approximately the same, indicating a nine-tenths loss in actual numbers in the soil for each sampling interval as compared with the preceding one; and this seems to hold true until the fortieth day. It may be explained that the moist series suffered much from the dropping of leaves heavily infected from the early samplings, and the resulting figures are somewhat erratic.

In the dry series there is a decided drop following the initial drying immediately after inoculation. Then follows a slow decline followed by an inexplicable increase between the tenth and thirty-fourth days. Afterwards there is an extremely gradual decline, if any, extending to the one hundred and sixty-sixth day.

On October 27, 1919, the one hundred and twelfth day of the test, a portion of the soil was removed from each of the three dry pots and moistened with sterile distilled water. The following tabulation shows the results of inoculation tests made from these moistened lots in comparison with the original dry soil. Two thousand punctures were inoculated from each lot of soil, making 6,000 for each test of moistened or of dry soil. The figures are total infections from 6,000 punctures.

	Date of sampling.						
	Oct. 29.	Oct. 31.	Nov. 3.	Nov. 5.	Nov. 7.	Nov. 10.	Nov. 17.
Dry soil.	2	6	3	8	9	0	6
Moistened soil.	0	0	0	0	0	0	0

The application of sterile distilled water seemingly resulted in prompt and complete extinction of *P. citri* in this dry soil which had constantly shown the presence of at least small numbers of the organism during almost four months. A repetition of the test, begun November 14, 1919, confirms these results.

That this extinction was not due to any toxic property peculiar to the distilled water was shown by a second test begun December 13, 1919, in which spring water and deep well water were used for wetting the soil. Tests on the third and seventh days were negative for all lots of moistened soil, while the dry soil continued to show the usual trace of *P. citri*.

That rate of drying would have an influence on the residuum of *P. citri* is to be expected and probably accounts for some of the irregularities

already noted in the behavior of the dry soil series when no control was exercised over the rate of drying. In a preliminary test, comparisons were made of the infective power of soil samples similarly inoculated and air-dried with different rates of rapidity at approximately the same rather warm temperature. A sample dried in less than one day gave 1.5 per cent infection, one dried in two days gave 0.1 per cent infection, and one dried in seven days gave no infection in tests made in each case immediately after drying.

An extended test of persistence in air-dry soil was made by Miss Clara H. Hasse. On October 22, 1918, soil from Winter Park, Fla., was heavily inoculated and dried as quickly as possible, in about one hour. Tests for infectiveness were made by several methods, usually by dusting the dry soil over punctured leaves which were atomized with water, the plants being later held in moist chambers. The total punctures inoculated in each test ranged from 600 to 5,000. The following percentage results were obtained:

	Date of sampling.									
	Oct. 22, 1918.	Oct. 25, 1918.	Oct. 28, 1918.	Nov. 1, 1918.	Nov. 8, 1918.	Jan. 2, 1919.	June 11, 1919.	Aug. 8, 1919.	Sept. 23, 1919.	Dec. 26, 1919.
Percentage of infection	94.1	68.3	8.5	2.3	1.5	0.1	0.24	0.22	0.48	0.05

On December 22, 1919, a portion of this soil was moistened with tap water from a deep well and was tested on the fourth and seventh days in comparison with the part remaining dry. In these tests the moistened soil gave no infection, while the dry soil continued to show traces.

PERSISTENCE IN SOILS MADE ARTIFICIALLY ALKALINE AND ACID

Greenhouse potting soil was used in 6-inch pots. Duplicate pots were watered each with 400 cc. of water containing 1.6 cc. sulphuric acid. Two pots were watered with 400 cc. of water containing 224 cc. clear lime water prepared by slaking 25 gm. quicklime and making up to 1,000 cc. A titration test showed this lime water to be sufficient to neutralize the quantity of acid applied to the other pots. A third pair of pots was watered with 400 cc. distilled water. After standing three days all pots were equally inoculated with *P. citri*. On each sampling date litmus paper tests of the 1/1 soil washings were made, and such small amounts of lime water or diluted acid were added as seemed necessary to maintain approximately the original distinct acidity in one set and distinct alkalinity in the other. The watering of all sets was equalized. The percentage results given in Table IX are based on infections out of 2,000 inoculated punctures.

TABLE IX.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of three soil solutions at various intervals after the soils had been treated with lime water, dilute sulphuric acid, and distilled water, respectively, and inoculated with *P. citri*

Number of days between inoculation and sampling.	1. Alkaline soil.				2. Normal soil.				3. Acid soil.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	80	95	70	33	93	100	46	26	16	80	50	24
2.....	69	84	67	49	83	70	46	23	7.5	22	3.8	1.5
4.....	56	46	38	8.9	41	40	30	3.0	.6	.5	.5	0
7.....	9.1	5.1	2.9	.6	3.8	2.1	.6	.2	0	0	0	0
9.....	2.7	.4	.2	.1	.5	.2	0	0	0	0	0	0
11.....	.1	0	0	.05	0	0	0	0	0	0	0	0
14.....	0	.3	0	0	.05	0	0	0	0	0	0	0
18.....	.2	.5	0	.05	0	0	0	.05	0	0	0	0
23.....	0	0	0	0	0	0	0	0	0	0	0	0
30.....	0	0	0	0	0	0
37.....	0	0	0
46.....	0	0	0

This preliminary and very artificial series indicates a slight retardation of decline in the alkaline soil and a distinct acceleration in the acid soil. In the latter, one notes the low infection percentages for the 1/1 dilution as compared with the 1/10 of the same series, or with the 1/1 of the other two series. While there is quite generally a tendency for the 1/1 dilution to give unexpectedly low results, the present instance suggests that the rather high acidity of the first wash water vehicle may play a part here in preventing infection. This matter calls for further experimentation. Since the tendency of most citrus soils is toward acidity, the evidence presented in Table IX is reassuring as to the decline of *P. citri* in such soils, notwithstanding the very unnatural conditions of the experiment.

PERSISTENCE DEEP IN THE SOIL

The tests for downward penetration were made by placing partially dry soil in open pasteboard cylinders 3 inches in diameter and watering the surface with a strong *P. citri* suspension until the whole was saturated. Sections were made at proper intervals and samples taken with proper precautions from the axis of the soil column. For vertical ascent the cylinders were placed in a shallow pan containing the suspension of *P. citri*.

In an 8-inch column of Florida sandy soil sampled at 2-inch intervals on November 20, 1918, downward penetration was shown to be very uniform throughout. A 15-inch column of greenhouse potting soil was tested October 1, 1919, with similar practically uniform penetration, as shown by sampling at 3-inch intervals.

In Florida soil tested for vertical ascent, the capillary rise was 6 inches during four hours. Two-inch samplings showed *P. citri* to be uniformly distributed.

While testing experimental methods, it was found that the organism is readily carried in the capillary current along an absorbent cotton wick at least 10 inches.

The indication that *P. citri* may readily penetrate deep into the soil raises the question of whether conditions deep in the soil may influence the persistence of the organism differently from those near the surface. A test was made by burying 4-inch pots of inoculated potting soil in large containers, so that the pots were completely surrounded by 8 inches of soil. Samplings were made at approximately 5-day intervals. No decided difference was noted between the buried pots and similarly inoculated ones held on the greenhouse bench.

PERSISTENCE IN AUTOCLAVED SOIL

Greenhouse potting soil in 4-inch pots was autoclaved July 15, 1918, for one hour with steam at 12 pounds pressure. When the soil was cold four autoclaved pots were inoculated, as well as four others containing similar soil not autoclaved. The percentage results in Table X are based on infection out of 1,200 inoculated punctures.

TABLE X.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of solutions from unautoclaved and autoclaved soils at various intervals after the soils had been inoculated with *P. citri*

Number of days between in- oculation and sampling.	Unautoclaved soil.				Autoclaved soil.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	73	73	16	0.9	44	3.4	6.2	0.3
4.....	60	35	7.8	3.7	60	68	13	15
9.....	0.9	0.7	.1	0	28	17	75	40
14.....	0	0	0	0	9.5	4.3	.8	.7
18.....	.1	.2	0	0	9.2	13	9.4	1.3
24.....	0	.1	0	0	1.9	1.9	2.1	.2
29.....	0	0	0	0	.8	.3	.3	.2
35.....	0	0	0	0	.4	.1	0	.1
44.....	0	0	0	0

The pots were kept on the greenhouse bench, each covered with paper. No special precautions were adopted to insure continued sterility in the autoclaved pots, if indeed the original steaming was sufficient for complete sterilization. Platings on agar at the end of the test showed miscellaneous bacteria in these pots in apparently as great numbers as in the unautoclaved ones. The autoclaved soil shows a decided lag in the decline of *P. citri*. A second series run two months later confirms this result.

PERSISTENCE IN WATER

Water was held in cotton-stoppered flasks in 200-cc. quantities. Water from a local spring was used in comparison with distilled water. Unfortunately the flasks of autoclaved distilled water became contaminated,

as was shown by Petri dish platings soon after the series was begun, and the results from them are not included in the tabulation. The percentages in Table XI are based on infection in 1,500 punctures.

TABLE XI.—Percentages of infection on grapefruit leaves inoculated with graded dilutions of distilled water, autoclaved spring water, and unautoclaved spring water at various intervals after the water had been inoculated with *P. citri*

Number of days between inoculation and sampling.	Distilled water.				Spring water, autoclaved.				Spring water, not autoclaved.			
	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000	1/1	1/10	1/100	1/1,000
0.....	100	97	43	14	97	87	51	8.2	100	96	58	13
2.....	0.1	0	0	0	72	8.2	5.8	.3	1.7	0.5	0.5	0
4.....	0	0	0	0	85	28	5.1	.8	0	0	0	0
7.....	0	0	0	90	69	12	0	0	0
11.....	0	0	58	25	0	0
16.....	0	34	0
21.....	0	44	0
30.....	0	40	0

Two other series confirmed the very rapid decline noted above in either distilled or ordinary surface water when it is nonsterile. Contrasted with this is the long persistence of moderately reduced numbers of the organism in the sterilized spring water.

The question is sharply raised, does autoclaving promote the persistence of *P. citri* in soil or water by destroying something that is deleterious or by producing something that is favorable? Autoclaving, in general, has its greatest effect in destroying the organic fauna and flora of the medium, and a subsidiary one in modifying the nutritive materials contained in it. The supposition that starvation may be the cause of the normal decline and that autoclaving the soil supplies enough available nutriment for a greatly prolonged persistence does not seem reasonable because of the disproportion between the changes that could possibly be brought about by autoclaving and the effects observed on *P. citri* persistence. Furthermore, this supposition of starvation is not adequate to explain the extinction of *P. citri* in air-dry soil when moistened.

INHIBITORS

The deleterious effects of organisms on the development of *P. citri* is frequently observed in poured plates when fungus or bacterial contaminants entirely inhibit the development of *P. citri* for considerable distances from their limits of growth. The behavior of some of these inhibitors has been made the subject of special preliminary study.

A series of plates was prepared September 13, 1919, from beef agar rather heavily and uniformly inoculated with *P. citri*. When hard, they were inoculated in addition with a bacterium, designated inhibitor A, previously obtained from a chance contamination on a poured plate. On some plates two streaks of the inhibitor were made at right angles

across the plates; in others it was planted at the center and at four spots near the circumference; in still others it was planted abundantly over the plate. Seven days later *P. citri* was seen growing in triangular areas between the limbs of the crosses, in isolated patches with concave borders between the spots, and not at all on the plates with numerous colonies of the inhibitor. It appeared only where the distance was at least 15 to 18 mm. from the nearest border of an inhibiting colony. A hand lens and the low power of the microscope brought within range of vision two successive graded zones each about 3 mm. wide of smaller *P. citri* colonies edging the clearly visible areas. The average distance from the edge of the inhibiting colony to the *P. citri* colonies of microscopic size was about 10 mm. Repeated attempts to cultivate *P. citri* from bits of agar from this clear 10-mm. zone failed, although the abundance of the original inoculation would have made it easy to recover the organism at any point, if it were still alive. It was recovered in culture from the microscopic and the clearly visible zones, and no extension of the killing effect could be determined after a further lapse of seven days, during which time there was no apparent growth of the inhibiting colonies.

The testing of some 40 miscellaneous soil bacteria and fungi on beef agar plates by the streak or the spot method showed about one-fourth of the number to have some degree of inhibiting effect, while three seemed to stimulate or accelerate the development of *P. citri* colonies, at least at the beginning of their development.

On other media the effects of certain of these inhibitors differed from those exhibited on beef agar, the inhibiting effect being reduced or entirely lost on certain media.

Tests in the soil itself must be conducted before definite statements can be made as to the part such potential inhibitors or destroyers actually play in the decline of *P. citri* under soil conditions. However, the hypothesis that the deleterious effects on *P. citri* are brought about by certain organisms in the soil is in harmony with the experimental evidence thus far obtained and seems to be a reasonable explanation of the phenomenon.

It seems reasonable to suppose that *P. citri* can persist in dry soil partly at least because of suspended activity of deleterious organisms, and that the addition of water makes possible a renewal of their unfavorable activity.

INFECTION OF GRAPEFRUIT ROOTS BY *P. CITRI*

The question naturally arises as to whether roots of citrus species are highly susceptible to citrus-canker infection. The following tests bear on this point.

On May 20, 1918, eight pots of soil were inoculated heavily with *P. citri* culture and planted with grapefruit seed, about 50 per pot. Eight other pots were similarly prepared without inoculation. In another set both seed and soil were inoculated, and in still another only the seeds were

inoculated. After two months the seedlings in all had made good growth, and there was no evidence of citrus-canker lesions on any part of the plants. In the light of present knowledge it would not have been expected that a single soil inoculation at the time of planting would persist long enough to become very effective.

On July 10, 1918, a series of pots was planted with grapefruit seed and given frequent waterings with *P. citri* suspension, on July 10, 13, 17, 20, 24, 27, and August 2. By this time the seedlings had emerged above ground, and before each subsequent watering several cuts were made through the soil with a knife to produce root wounds. Further applications of *P. citri* suspension were made August 5, 8, 10, 14, and 16. On August 31 the seedlings were removed, washed, and examined with a hand magnifier. No canker lesions were apparent on any part of the 40 plants thus examined. A test performed on grapefruit leaves with soil from these pots which had received 12 applications of heavy inoculum at close intervals gave negative results.

Direct inoculation of the roots of potted grapefruit seedlings was made as follows: On July 27, 1918, potted plants were selected with vigorous roots of about $\frac{3}{16}$ inch diameter extending $\frac{1}{2}$ to 3 inches through the drainage holes of the pots. These roots were punctured at 10 points each, wrapped in cotton wet with *P. citri* suspension, and later placed in flats of moist, clean sand. Two weeks later infection was 40 per cent. Microscopic sections showed typical canker lesions involving the cortex. Pure cultures of *P. citri* were readily obtained by plating, and grapefruit leaves were infected therefrom. Four months later no extension of infection was apparent on the roots, most of them having continued their growth to all appearance normally. In several, however, the roots were broken at old lesions apparently following secondary decay. The plants as a whole had not suffered.

The indications are that young grapefruit roots are not readily infected except through direct wound inoculation and that the plants do not suffer from a moderate number of lesions so produced.

SUMMARY

(1) The method of using graded dilutions of soil washings for inoculating punctured grapefruit leaves proved satisfactory for indicating the relative abundance of *P. citri* in the soil at times of sampling.

(2) Tests on many types of soil, including representative ones from citrus regions, show a very rapid decline of *P. citri* in all.

(3) This decline was retarded slightly by rendering the soil alkaline with lime water or by lowering its temperature, and more decidedly by withholding water or by previous sterilizing with steam.

(4) An extremely long persistence, in very small numbers, is noted in soil held in air-dry condition; but the organism seemingly suffers prompt extinction when water is again added.

(5) The decline is accelerated decidedly by the addition of dilute sulphuric acid or by a moderate rise in temperature.

(6) *P. citri* may easily penetrate the soil to depths ordinarily cultivated, but the normal decline seems to occur at such depths.

(7) In water the decline is more rapid than in soil. Previous sterilizing of the water has a decided effect in prolonging persistence.

(8) Certain bacteria found commonly in soils have a marked deleterious effect on *P. citri* in artificial culture media both by inhibiting growth and by killing.

(9) The presence of such deleterious organisms in soils would probably be concerned in producing a decline of *P. citri*.

(10) Young roots of grapefruit seedlings seem not to be readily infected by *P. citri* except through wounds.

CONCLUSION

The main question at issue is whether or not *P. citri* can persist in the soil to a sufficient degree or for a long enough time to be a source of danger in the dissemination or holding over of the citrus-canker disease. The experimental evidence shows clearly that the organism undergoes a rapid and continuous decline in numbers under soil conditions that would obtain in agricultural practice. As a rule, this decline reaches the vanishing point for *P. citri* in about two weeks by the test methods employed, and it is only reasonable to suppose that the downward trend continues rapidly in such cases to absolute extinction. The potential ability of certain soil organisms to destroy *P. citri*, as shown in certain artificial culture media, lends weight to this latter supposition. Even where long-time persistence has been induced experimentally, the conditions necessary to bring it about are too extreme to make a duplication probable under natural conditions. Furthermore, the experimental methods employed for testing the infectiveness of the soil are many times more severe than would obtain under most favorable natural conditions for the spread of infection from soil to plants. All these considerations suggest that agricultural soils probably can not long retain a dangerous possibility of disseminating the citrus-canker organism.

VARIATION OF INDIVIDUAL PIGS IN ECONOMY OF GAIN¹

By R. C. ASHBY and A. W. MALCOMSON, *Division of Animal Husbandry, Minnesota Agricultural Experiment Station*²

When the initial tests with self-feeders were undertaken in 1914 the question at once arose, "What variations will appear in rations as selected by individual pigs?" To answer it 10 pigs were self-fed individually during the summer of 1915. A study of their rations has been published.³ But in tabulating the data another factor presented itself—namely, material variations in economy of gain by the different individuals. Two similar tests have been continued in order to gain further information on this point. It is our purpose to report here the results thus far obtained.

While marked variations have been found with all groups tested, no attempt is made to explain them, because facilities have been entirely inadequate to permit a fundamental study. In one instance the junior author has made thorough type and conformation studies of 15 individuals. His data will appear in thesis form.

To date 67 individuals, representing 14 litters, have been fed individually. The experiments have been conducted during three summers and are reported as tests A, B, and C. As explained later this report includes the data on 63 pigs.

TEST A, FEEDING PERIOD 128 DAYS

As mentioned, the records of the pigs fed in 1915 are already available. A summary for nine pigs is presented here, No. 11 being omitted because of its low final weight. For the nine pigs the average initial weight was 47.42 pounds and the average final weight 267.33 pounds. A comparison of the pigs is given in Table I.

Classified according to the degree of variation from the mean or normal grain requirement for the group:

- 1 pig shows a variation from the mean of more than 10 per cent.
- 2 pigs show a variation from the mean of between 5 and 10 per cent.
- 6 pigs show a variation from the mean of less than 5 per cent.

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² The authors express to Prof. H. K. Hayes their appreciation for assistance in arranging and verifying the correlation table, and to Dr. C. W. Gay and Miss Alice McFeely for helpful suggestions.

³ ASHBY, R. C. SELF-BALANCED RATIONS BY INDIVIDUAL PIGS. *In Amer. Soc. Anim. Prod. Proc.* 1915/16 p. 197-209, illus. 1917.

TABLE I.—Grain required to produce 100 pounds gain in pigs of test A
[Average initial weight, 47.42 pounds; average final weight, 267.33 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variation from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
I.....	4	1.908	402.04	+ 7.24	0.183
I.....	2	1.890	380.21	-14.59	3.695
I.....	4	2.063	376.71	-18.09	4.582
I.....	6	1.492	423.04	+28.24	7.152
I.....	7	1.668	404.38	+ 9.58	2.426
2.....	10	1.603	430.76	+35.96	9.108
2.....	12	1.369	397.99	+ 3.19	.808
2.....	13	1.635	395.60	+ .80	.202
2.....	14	1.835	355.31	-39.49	10.002
Mean grain for 100 pounds gain.....			394.80		

TEST B, FEEDING PERIODS 84 AND 100 DAYS

In 1916, 26 pigs were fed individually. Six of these which were fed on pasture plots make up group 1. Of the remaining 20, 7 which averaged 193.71 pounds each at the close of the test constitute group 2. The remaining 13 were younger pigs, except DJ 37 and P 72, which started on feed at lighter weights and averaged only 137 pounds at the close. These 13 made up group 3. The data of test B are given in Tables II to IV.

TABLE II.—Grain required to produce 100 pounds gain in pasture-fed pigs of test B, group 1

[Average initial weight, 34.2 pounds; average final weight, 156.9 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variation from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
DJ.....	33	1.357	371.70	+28.59	8.332
DJ.....	35	1.514	346.20	+ 3.09	.900
PY.....	3	1.315	289.20	-53.91	15.712
DJ.....	46	1.294	365.80	+22.69	6.613
PD.....	2	1.250	325.00	-18.11	5.278
PD.....	6	1.238	364.10	+20.99	6.117
Mean grain for 100 pounds gain.....			343.11		

TABLE III.—Grain required to produce 100 pounds gain in dry-lot pigs of test B, group 2

[Average initial weight, 42.07 pounds; average final weight, 193.71 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variation from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
DJ.....	30	1.417	455.20	+46.85	11.473
DJ.....	31	1.080	456.30	+47.95	11.742
DJ.....	32	1.900	425.00	+16.65	4.070
DJ.....	34	1.287	408.80	+ .45	.110
DJ.....	36	1.940	382.30	-26.05	6.379
PY.....	5	1.577	367.20	-41.15	10.077
PY.....	6	1.724	383.40	-24.95	6.109
Mean grain for 100 pounds gain.....			408.35		

TABLE IV.—Grain required to produce 100 pounds gain in dry-lot pigs of test B, group 3
[Average initial weight, 29.6 pounds; average final weight, 137 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variations from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
DJ.....	38	1. 380	361. 70	—18. 09	4. 763
DJ.....	39	1. 326	379. 20	— . 59	. 155
DJ.....	41	1. 258	399. 60	+19. 81	5. 216
DJ.....	42	1. 198	392. 20	+12. 41	3. 267
DJ.....	43	1. 397	406. 10	+26. 31	6. 927
DJ.....	44	1. 040	383. 10	+ 3. 31	. 871
PD.....	1	1. 452	363. 30	—16. 49	4. 341
PD.....	3	1. 282	392. 30	+12. 51	3. 293
PD.....	4	1. 052	378. 20	— 1. 59	. 418
PD.....	5	1. 175	431. 40	+51. 61	13. 589
DJ.....	37	1. 157	358. 70	—21. 09	5. 553
PY.....	2	1. 177	340. 30	—39. 40	10. 307
Mean grain for 100 pounds grain.....			379. 79		

If the three groups are combined, the pigs may be classified as follows on the basis of degree of variation from their respective means:

- 6 pigs show a variation from the mean of more than 10 per cent.
- 9 pigs show a variation from the mean of between 5 and 10 per cent.
- 10 pigs show a variation from the mean of less than 5 per cent.

TEST C

In 1917 three tests were conducted. As before, 6 pigs were fed on pasture and 9 were carried on individual self-feeders in dry lot. In addition 16 pure-bred pigs intended for breeding animals were selected for individual feeding. Of the 15 market pigs 2 were very small at the beginning of the test and much lighter than the others at the close. For that reason they are omitted. The data for the three groups are given in Tables V to VII.

TABLE V.—Grain required to produce 100 pounds gain in pasture-fed pigs of test C, group 1, fed 118 days
[Average initial weight, 35.8 pounds; average final weight, 172.84 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variations from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
PB.....	6	1. 030	419. 98	+49. 81	13. 457
PB.....	7	1. 118	364. 84	— 5. 31	1. 433
PB.....	16	1. 239	365. 26	— 4. 90	1. 324
PY.....	1	1. 220	386. 25	+16. 08	4. 345
PY.....	4	1. 197	320. 94	—49. 21	14. 296
Mean grain for 100 pounds gain.....			370. 16		

TABLE VI.—Grain required to produce 100 pounds gain in dry-lot pigs of test C, group 2, fed 118 days

[Average initial weight, 43.62 pounds; average final weight, 185.46 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variations from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
PB.....	1	1.42	420.91	+36.82	9.586
PB.....	3	1.06	473.57	+89.48	23.296
PB.....	9	1.26	363.43	-20.66	5.378
PB.....	15	1.15	343.85	-40.24	10.476
DJ.....	12	1.47	370.85	-13.24	3.447
DJ.....	13	1.05	419.12	+35.03	9.120
PY.....	2	1.11	340.13	-43.96	11.445
PY.....	3	1.05	342.31	-41.78	10.877
Mean grain for 100 pounds gain.....			384.09		

The degrees of variation from the group means classify thus:

6 pigs show a variation from the mean of more than 10 per cent.

3 pigs show a variation from the mean of between 5 and 10 per cent.

4 pigs show a variation from the mean of less than 5 per cent.

TABLE VII.—Grain required to produce 100 pounds gain in pasture-fed pigs of test C, group 3, fed 79 days

[Average initial weight, 63.6 pounds; average final weight, 147.3 pounds]

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.	Variations from mean grain for 100 pounds gain.	
		Pounds.	Pounds.	Pounds.	Per cent.
PC.....	2	0.94	309.78	-55.18	15.119
PC.....	3	1.01	394.50	+29.54	8.093
PC.....	6	.88	321.42	-43.54	11.931
PC.....	8	1.06	315.89	-49.07	13.445
DJ.....	2	1.15	332.96	-32.00	8.768
DJ.....	3	1.18	396.15	+31.19	8.546
DJ.....	5	1.06	394.40	+29.44	8.066
DJ.....	6	.81	327.81	-37.15	10.170
DJ.....	7	1.21	367.91	+2.95	.808
DJ.....	8	1.45	335.39	-29.57	8.102
DJ.....	10	1.35	461.57	+96.61	26.471
DJ.....	11	1.14	308.81	-56.15	15.385
PC.....	13	.84	442.83	+77.87	21.336
PC.....	14	.83	451.73	+86.77	23.775
PC.....	15	1.09	309.93	-55.03	15.079
PC.....	16	.89	362.25	-2.71	.742
Mean grain for 100 pounds gain.....			364.96		

Note that both extremes are found in the same litter, DJ 10 and DJ 11. Wide variations appear here, but because of the comparatively short feeding period of 79 days and the low final average weight these results can not be accepted on a par with those from the preceding groups. Tabulating the results for group 3 on the basis of extent of variation from the mean, we have:

- 3 pigs showing a variation from the mean of more than 20 per cent.
- 3 pigs showing a variation from the mean of between 15 and 20 per cent.
- 3 pigs showing a variation from the mean of between 10 and 15 per cent.
- 5 pigs showing a variation from the mean of between 5 and 10 per cent.
- * 2 pigs showing a variation from the mean of less than 5 per cent.

The occurrence and scope of variation are further emphasized by Table VIII in which the extremes from 11 litters are compared.

TABLE VIII.—*Extremes of daily gain and weight of grain required to produce 100 pounds gain in 11 litters.*

Litter.	Pig No.	Daily gain.	Grain for 100 pounds gain.
		<i>Pounds.</i>	<i>Pounds.</i>
I.....	4	2. 06	376. 71
I.....	6	1. 49	423. 04
2.....	10	1. 60	430. 76
2.....	14	1. 83	355. 31
PD.....	2	1. 25	325. 00
PD.....	6	1. 23	364. 10
DJ.....	30	1. 41	455. 20
DJ.....	36	1. 94	382. 30
DJ.....	38	1. 38	361. 70
DJ.....	43	1. 39	406. 10
PY.....	1	1. 22	386. 25
PY.....	4	1. 19	320. 94
DJ.....	12	1. 47	370. 85
DJ.....	13	1. 05	419. 12
PC.....	2	. 94	309. 78
PC.....	3	1. 01	394. 50
PC.....	14	. 83	451. 73
PC.....	15	1. 09	309. 93
DJ.....	3	1. 18	396. 15
DJ.....	6	. 81	327. 81
DJ.....	10	1. 35	461. 51
DJ.....	11	1. 14	308. 81

Of the 65 pigs an unexpectedly large number show marked variation from the normal or mean grain requirement per unit of gain.

Summing up all groups, we find:

- 22 pigs showing a variation from the mean of more than 10 per cent.
- 19 pigs showing a variation from the mean of between 5 and 10 per cent.
- 22 pigs showing a variation from the mean of less than 5 per cent.

On a percentage basis:

- 34.92 per cent exceeded 10 per cent variation.
- 30.15 per cent showed between 5 and 10 per cent variation.
- 34.92 per cent showed less than 5 per cent variation.

As stated before, no attempt is now made to explain these differing requirements, but the question of a possible correlation between the rate of gain and economy of gain naturally suggests itself. In fact, a casual inspection of the groups leads one to expect such a correlation.

In Tables IX to XV the individuals are ranked in order of efficiency both as to daily rate of gain and economy of gain.

TABLE IX.—*Rank of pigs of test A in rate and economy of gain*

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	4	1.....	14	6.....	13	6.....	1
2.....	1	2.....	4	7.....	10	7.....	7
3.....	2	3.....	2	8.....	6	8.....	6
4.....	14	4.....	13	9.....	12	9.....	10
5.....	7	5.....	12				

TABLE X.—*Rank of pigs of test B, group 1, in rate and economy of gain*

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	DJ 35	1.....	PY 3	4.....	DJ 46	4.....	PD 6
2.....	DJ 33	2.....	PD 2	5.....	PD 2	5.....	DJ 46
3.....	PY 3	3.....	DJ 35	6.....	PD 6	6.....	DJ 33

TABLE XI.—*Rank of pigs of test B, group 2, in rate and economy of gain*

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	DJ 36	1.....	PY 5	5.....	DJ 30	5.....	DJ 32
2.....	DJ 32	2.....	DJ 36	6.....	DJ 34	6.....	DJ 30
3.....	PY 6	3.....	PY 6	7.....	DJ 31	7.....	DJ 31
4.....	PY 5	4.....	DJ 34				

TABLE XII.—*Rank of pigs of test B, group 3, in rate and economy of gain*

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	PD 1	1.....	PY 2	7.....	DJ 42	7.....	DJ 44
2.....	DJ 43	2.....	DJ 37	8.....	PY 2	8.....	DJ 42
3.....	DJ 38	3.....	DJ 38	9.....	PD 5	9.....	PD 3
4.....	DJ 39	4.....	PD 1	10.....	DJ 37	10.....	DJ 41
5.....	PD 3	5.....	PD 4	11.....	PD 4	11.....	DJ 43
6.....	DJ 41	6.....	DJ 39	12.....	DJ 44	12.....	PY 5

TABLE XIII.—Rank of pigs of test C, group 1, in rate and economy of gain

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	PB 16	1.....	PY 4	4.....	PB 7	4.....	PY 1
2.....	PY 1	2.....	PB 7	5.....	PB 6	5.....	PB 6
3.....	PY 4	3.....	PB 16				

TABLE XIV.—Rank of pigs of test C, group 2, in rate and economy of gain

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	DJ 12	1.....	PY 2	5.....	PY 2	5.....	DJ 5
2.....	PB 1	2.....	PY 3	6.....	PB 3	6.....	DJ 13
3.....	PB 9	3.....	PB 15	7.....	PY 3	7.....	PB 1
4.....	PB 15	4.....	PB 9	8.....	DJ 13	8.....	PB 8

TABLE XV.—Rank of pigs of test C, group 3, in rate and economy of gain

Rate of gain.		Economy of gain.		Rate of gain.		Economy of gain.	
Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.	Rank.	Pig No.
1.....	DJ 8	1.....	DJ 11	9.....	DJ 5	9.....	PC 16
2.....	DJ 10	2.....	PC 2	10.....	PC 3	10.....	DJ 7
3.....	DJ 7	3.....	PC 15	11.....	PC 2	11.....	DJ 5
4.....	PJ 3	4.....	PC 8	12.....	PC 16	12.....	PP 3
5.....	DJ 2	5.....	PC 6	13.....	PC 6	13.....	DJ 3
6.....	DJ 11	6.....	DJ 6	14.....	PC 13	14.....	PC 13
7.....	PC 15	7.....	DJ 2	15.....	PC 14	15.....	PC 14
8.....	PC 8	8.....	DJ 8	16.....	DJ 6	16.....	DJ 10

Selecting approximately the top half of each group, on the basis of rate of gain, we have 4 top pigs from test A; 3 from test B, group 1; 3 from test B, group 2; 6 from test B, group 3; 3 from test C, group 1; 4 from test C, group 2; and 8 from test C, group 1; making a total of 31 pigs. Of this number 19 were placed in the corresponding top halves of their respective economy columns.

In other words, slightly more than 60 per cent of the fastest-growing pigs were also distinctly economical producers. This would indicate that slightly more than one-half of the fastest-growing pigs in an average group would qualify on an economy basis.

The foregoing comparison is independent of litter relationships. Selecting and comparing the fastest-growing pig with the slowest-gainer from the same litter, we find the following results from 12 litters:

- In 6 cases the fastest growing pig was most economical.
- In 3 cases the fastest growing pig was least economical.

- In 3 cases the fastest growing pig was moderately economical.
- In 2 cases the slowest-growing pig was most economical.
- In 5 cases the slowest-growing pig was least economical.
- In 5 cases the slowest-growing pig was moderately economical.

Apparently this indicates a certain degree of correlation between the characters under discussion. As a more accurate determination of correlation between rate of gain and economy of gain the data are correlated in Table XVI. For this purpose the variations, both in rate of gain and economy of gain, are reduced to a percentage basis.

XVI.—Correlation between rate of gain and economy of gain

		Rate of gain (in percentages of the mean).														Total.
Economy of gain (in percentages of the mean.)		70	75	80	85	90	95	100	105	110	115	120	125	130	135	
	85.....					1	...	2	2	1	6
	90.....		1		1	1	3	1	1	1	1	10
	95.....					...	2	...	1	3	1	1	2	10
	100.....			2	3	...	3	...	2	1	2	13
	105.....				1	...	3	1	2	1	...	1	9
	110.....	1				2	2	2	...	1	...	1	9
	115.....					1	1	2
	120.....			1												1
	125.....			1	...	1	...							1	...	3
	Total.....	1	1	4	5	6	14	6	8	8	3	3	2	1	1	...

r= -0.452±0.068.

The resultant coefficient of correlation ($r = -0.452 \pm 0.068$) shows a distinct negative correlation between rate of gain and economy of gain, entirely disproving the apparent relation shown by Tables IX to XV. The differing requirements per unit of gain are of much practical moment. As has been noted, the variation in rate of gain shows a standard deviation in percentage of 9.57 ± 0.58 and an average deviation of 8.01 per cent.

POSSIBLE APPLICATION

Pointing out applications before establishing final conclusions is as dangerous as selling property without possession of title, but a consideration of probabilities is ever in order.

It is safe to emphasize again the danger of conclusions based on feeding trials where small groups are the experimental units. If average individual variations of 7 per cent are at all common, a statement in a former Oregon Experiment Station bulletin¹ that—

the reader should therefore hesitate at putting too much weight on differences amounting to less than 10 per cent carries much weight.

¹ WITHYCOMBE, James, POTTER, Ermine L., and SAMSON, George R. EXPERIMENTS IN SWINE FEEDING. Oreg. Agr. Exp. Sta. Bul. 127, p. 5. 1915.

However, our main interest lies in the possibility of utilizing this factor of variation, making it a definite factor in the breeder's support. Is it a hereditary character? How is it transmitted? Can the breeder through careful testing and selective mating develop or produce a strain that is more economical in feeding or pure for the quality of economy in production? Can he produce a line that is homozygous for this characteristic? Extreme results are not to be expected, but even a moderate saving, if constant, would be a marked achievement.

In this connection a feature of Danish agricultural practice is very interesting. An article¹ describing it came to hand as our data were being tabulated, and a brief quotation is pertinent in this connection:

There is, however, quite another group of qualities which must be kept in mind in connection with swine-breeding, but which cannot be estimated with sufficient accuracy with the naked eye, namely, the quality of the bacon and the thrivingness and growing energy of the pigs.

The Experimental Laboratory has, during a long period of years, carried out experiments with regard to the offspring of stud animals in the breeding centers which afford reliable and helpful information as to the powers of transmission of qualities possessed by the stud animals in regard to the qualities mentioned. It is the breeding centers which supply the material for these experiments.

The owner of each recognized breeding center is bound to supply on an average two young pigs from selected sow annually to the Experiment Stations, and as there are about 900 selected sows (757 Danish and 147 Yorkshire), the stations have at their disposal a good deal of material. For pecuniary and other reasons they have found it necessary to confine themselves to about 1,000 test animals per annum. Nevertheless, the experiments are on a big scale such as is scarcely equalled elsewhere.

The young pigs are supplied at the age of seven or eight weeks. Each experiment pen contains four full-blooded sisters and brothers. All the pens receive the same food mixture in weighed proportions, and the animals themselves are weighed at regular intervals. The experiments finish when the abattoir weight is reached . . . The result is made use of in the selection of stud animals, those being preferred whose descendants have shown the highest degree of thrivingness and growth energy and the best bacon.

This is a good plan and doubtless characteristic of the results obtained through Danish agricultural cooperative organization, though just how each pen could contain four litter mates when only two pigs are sent from each litter is a bit puzzling.

Of recent years the possibility of a "register of merit" for meat animals has received considerable attention. If more thorough investigation corroborates our results and should it be found possible to develop families or strains that are more economical producers, no sounder basis of preferment could be desired.

If selection along this line will achieve results, we believe it desirable to put the work on an individual basis from the start. The Danish plan deals with pen averages which our data show to be somewhat unreliable so far as indicating the true performance of the individuals concerned.

¹ MÖRKEBERG, Peter Aug. THE PRESENT POSITION AND FUTURE PROSPECTS OF SWINEBREEDING IN DENMARK. In Dept. Agr. and Tech. Instr. Ireland Jour., v. 17, no. 1, p. 46-47. 1916.

But since the "breeding centers" have been a factor for at least 20 years, and doubtless the "experimental laboratory" has been in operation a good part of that time, the continuation of the plan attests its efficiency. By adopting the individual as the unit we eliminate the probable inaccuracy of pen averages and hope to have taken at least one step in devising a practical method for measuring the efficiency of meat-producing animals.

PRODUCTION OF CONIDIA IN *GIBBERELLA* *SAUBINETII*¹

By JAMES G. DICKSON, *Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and Assistant Professor of Plant Pathology, University of Wisconsin, and HELEN JOHANN, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*.

The scab fungus *Gibberella saubinetii* (Mont.) Sacc., which attacks wheat, corn, rye, barley, and oats, has been considered as having a vegetative stage and two spore stages. The conidial and perithecial development terminates the active vegetative period. Strains producing abundant perithecia have been described as developing only a few conidia in scattered, sporodochia-like masses.

Cultural studies with a large number of strains of *G. saubinetii* show that in nature, as well as in artificial culture, this species produces conidia at two different periods during its development. Wollenweber² suggests this when he states that—

on steamed potato tuber the conidia form a short-lived pionnotes. The conidia of this pionnotes rapidly swell, separate into cells, germinate, and produce new conidia, which anastomose and form a stroma, while in the other species mentioned the conidia remain perfect, dry out, and are long-lived.

The first period of conidial production is in connection with the early mycelial growth of the culture, while the second occurs at the termination of the vigorous vegetative development. These later conidia are produced in definite sporodochia and are the only conidia generally described for this species. The production of perithecia is the final stage in the development of the culture.

During the summer of 1919, single-spore cultures were made by the authors from sporodochial conidia and ascospores taken from stock cultures and from wheat heads, wheat culms, and cornstalks collected in the field. These specimens were obtained from a number of widely separated points in the central and eastern States. Spores from all sources were placed in hanging drops of distilled water and sterile tap water, on poured plates of potato-dextrose agar and soil decoction agar, and on sterile soil. The subsequent development of the fungus was then studied at frequent intervals.

¹ The investigations upon which this paper is based were conducted as a cooperative project between the Office of Cereal Investigations of the Bureau of Plant Industry and the Wisconsin Agricultural Experiment Station.

² WOLLENWEBER, H. W. IDENTIFICATION OF SPECIES OF *FUSARIUM* OCCURRING ON THE SWEET POTATO, *IPOMOEA BATATAS*. In Jour. Agr. Research, v. 2, no. 4, p. 278. 1914.

The spores, both conidia and ascospores, behaved alike in germination. They germinated, as described by Wollenweber, by imbibing water, increasing the number of septa (fig. 1, A, C), and forming several mycelial strands from the different cells (fig. 1, C). When the cultures were grown in a saturated atmosphere, conidia were cut off from lateral branches of mycelial strands in 24 hours (fig. 1, B, D). In 48 hours a copious conidial production took place in definite sporodochia-like clusters (fig. 1, E). On extremely moist plates these clumps occasionally massed together to form a pionnotes. As mycelial development

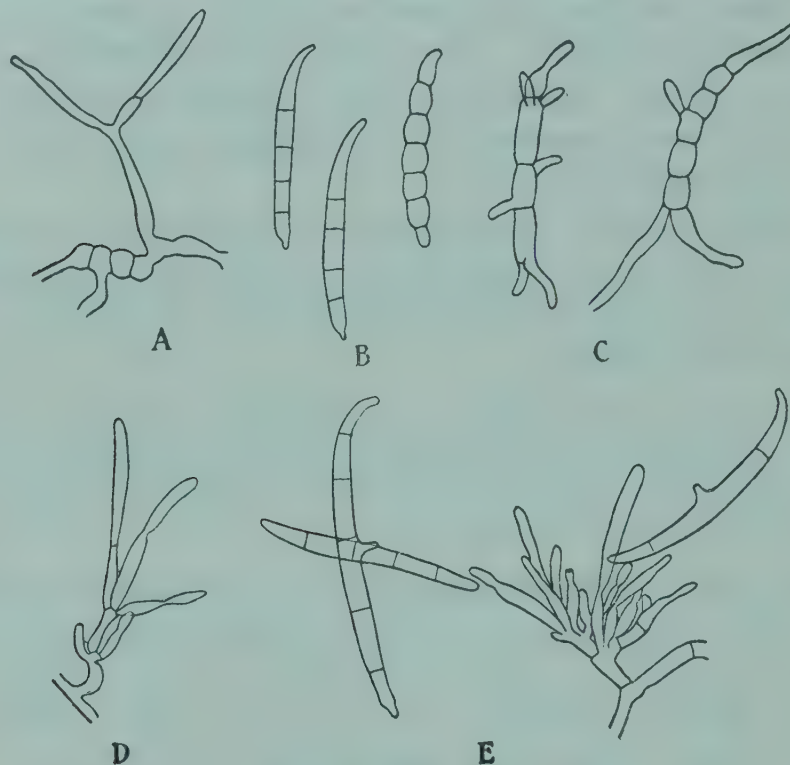


FIG. 1.—Conidial production in *Gibberella saubinetii* (Mont.) Sacc.: A, Ascospores from cornstalk, germinated in distilled water, producing conidia in three days; B, D, typical conidia and conidiophore from a 28-hour-old hanging drop culture from a conidium from A; C, germinating conidia from a 52-hour-old plate culture; E, conidiophore and germinating conidia from a 47-hour-old colony in a Van Tieghem cell. This colony was three generations from an ascospore. Potato-dextrose agar acidified with lactic acid was used unless otherwise stated.

progressed, new conidial masses developed and thus gradually increased the size of the pionnotes.

The conidia were pushed off the conidiophore before septation was completed, and new conidia formed in their place (fig. 1, E). Septation was completed after the conidia had been separated from the conidiophore. The conidia became swollen, septation increased, and germination took place in from 6 to 12 hours after leaving the conidiophore (fig. 1, B, C, E). When the cultures were moderately crowded and moisture and temperature conditions were suitable, all these conidia germinated, forming a stroma; and conidia development ceased until the final development of sporodochial conidia several weeks later. If, however, the conidia were transferred to a suitable medium and were not

overcrowded, they germinated, forming hyphae which bore masses of conidia within two days as previously described for the sporodochial conidia and ascospores. This conidial production went on indefinitely, if the culture did not dry or become crowded. The ninth generation of conidia from a single ascospore was produced in 20 days by transferring each successive generation to new plates of potato-dextrose agar. These conidia were produced only when the spores were transferred to a favorable medium and kept in a moist, warm atmosphere. When the temperature was lowered or when the culture became dry the conidia did not germinate but remained inert on the surface of the culture. Spores kept in this manner were rather resistant to both desiccation and low temperatures. Germination was obtained after several weeks' storage at temperatures of about 3° to 4° C., as well as when stored under dry conditions at room temperature.

Conidia were produced in two days from mycelium plated from infected root and stem tissues as well as from plated conidia and ascospores. Tissues infected with *G. saubinetii* were surface-sterilized and placed on potato-dextrose agar in poured plates. Conidia appeared on the developing mycelium two days after plating and were present in conspicuous sporodochia-like masses the third day. These conidia were identical with those formed on the mycelium from either ascospores or conidia.

The conidia formed during the vegetative development were 4 to 5 septate (fig. 1, B, E) and were of the same shape and size as the sporodochial conidia.

Inoculations on wheat plants showed that these conidia were as virulent in producing scab on wheat as were either sporodochial conidia or the vegetative mycelium. The spores germinated and caused infection within the same temperature range as the sporodochial conidia.

The work here reported shows that repeated crops of conidia of *G. saubinetii* can be produced in abundance in short periods of time from ascospores, sporodochial conidia, vegetative conidia, or mycelium, when favorable moisture and temperature conditions obtain. This ability of the wheatscab organism to produce virulent spores in abundance in short periods of time has an important bearing on the development of wheatscab epidemics.

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EFFECT OF MANURE-SULPHUR COMPOSTS UPON THE AVAILABILITY OF THE POTASSIUM OF GREENSAND

By A. G. McCALL, *In Charge of Soil Investigations*, and A. M. SMITH, *Chemist, Soil Investigations, Maryland Agricultural Experiment Station*

INTRODUCTION

The greensands and the greensand marl deposits of the eastern United States have long been regarded as a possible source of potassium for agricultural purposes. The literature of the last half of the nineteenth century contains many reports of the success that has followed the application of greensand marls to soils in Maryland, New Jersey, and other eastern States. Since many of these marls contain a high percentage of calcium carbonate, it is probable that the good results that followed their use was due in many cases to their lime content rather than to the potassium which they contained.

During the continuance of the war with Germany, the scarcity and the consequent high price of readily soluble potassium salts has served to direct attention in this country to the possibility of utilizing for agricultural purposes the potassium of these greensand deposits and has indicated the desirability of devising some efficient method of treatment that would render the potassium more available. At the suggestion of the fertilizer committee of the National Research Council, the Department of Soil Investigations of this Station has studied the effect of composting greensand with sulphur, manure, and other materials with a view to making available the potassium contained in the greensand. It is the purpose of this paper to report the results of this investigation.

HISTORICAL

As early as 1830 Thomas Gordon called attention to the great benefits that farmers in New Jersey were deriving from the use of marl. In a geological report published in 1868, Cook (6)¹ gives the analyses of a number of samples of marl from New Jersey and states that the use of this material has raised the land from a low state of exhaustion to a high stage of agricultural development. He states that some of these marls are so acid that heavy applications of as much as 50 tons to the acre

¹ Reference is made by number (*italic*) to "Literature cited," p. 255-256.

have been known to destroy all vegetation and advises that the use of such marls should be confined to well-limed land or that they should be composted with lime before being applied. In 1906 Patterson (12) published the results of the examination of 95 samples of Maryland marl. In summing up the results of his experimental work covering a period of 11 years this writer concludes that the shell marls of Maryland have very little commercial value because of the great bulk of worthless material contained in them but that they should have considerable local agricultural value, both as a source of lime and also for the potassium which they contain. He concludes that while much of the potassium in marls will become slowly available to plants through weathering, the change necessary to liberate the potassium could readily be brought about by burning the calcarious marls and slaking the product.

In a popular discussion of the agricultural value of greensand marl Blair (3) concludes that since potassium is of especial value to grass and to potatoes, the striking benefits derived from the use of marl on these crops would lead to the belief that such crops can use the potassium of the marl to a considerable extent.

From pot experiments carried out with crushed quartz and Shive's cultural solution as a basis, True and Geise (13, p. 492) conclude that—greensands and greensand marls from Virginia and New Jersey are able to supply sufficient potassium to satisfy the demands of Turkey Red wheat and red clover during the first two months of their growth.

They secured a greater dry weight of tops from cultures containing greensand marl than from those in which the potassium demand was supplied by potassium chlorid, potassium sulphate, or potassium phosphate. These results are in harmony with those reported by Lipman and Blair (8) who found that soybean plants fertilized with greensand produced as great a yield of hay as those receiving an application of soluble potassium salts, although the former failed to produce seed. These last-mentioned authors hold that their results seem to furnish proof of the ease with which the soybean gets its potash from slowly available sources up to the time the beans are forming and maturing. In the same report these writers describe another experiment in which Canada field peas and soybeans growing in sand cultures were given a general fertilizer treatment to which was added marl containing 6.5 per cent of potash. Two pots in this series received 20 gm. of marl, while two additional pots received in addition to the 20 gm. of marl, 3 gm. of sulphur each, with the thought that the oxidation of the sulphur might result in making more of the potash of the marl available. The Canada field peas were grown as the first crop, followed by the soybeans as a second crop. Both pots receiving the sulphur treatment gave very much decreased yields of field peas, and in one of the duplicates the soybeans that followed the peas failed completely. The other duplicate, however, gave a yield of soybeans slightly in excess of that produced by any of the other treat-

ments, including the pots receiving 2 gm. of potassium chlorid. In their conclusions they suggest—

the possibility of utilizing the potash of greensand marl and the potash of natural soil materials by growing soybeans and possibly certain other crops, which could be returned to the soil and thus furnish available potash for those crops which can not readily utilize potash from these natural sources.

Lipman, McLean, and Lint (10) composted 100-gm. portions of sea sand, sassafras loam, and greenhouse soil with manure, sulphur, and floats. At the end of 30 weeks analyses for water-soluble phosphoric acid showed increases in all the mixtures to which both sulphur and floats had been added. In one case 85 per cent of the total phosphorus in the floats had been made available, the increase in available phosphorus paralleling the oxidation of the sulphur as measured in terms of sulphates. In experiments conducted under field conditions, two of these authors (9) have shown that the sulphur-floats-soil compost may be utilized in making available the phosphorus of floats or raw ground phosphate rock. They suggest that this compost could be employed to advantage as a substitute for acid phosphate. Further studies at the New Jersey Experiment Station by McLean (11) led to the conclusion that the most economical combination for the production of available phosphoric acid is a compost composed of 100 parts soil, 120 parts sulphur, and 400 parts floats.

Brown and Warner (5) found that by composting floats with manure and sulphur it was possible to obtain a remarkable increase in the amount of available phosphoric acid. The increase was greater where the sulphur and floats were intimately mixed with the manure than where the material was arranged in alternate layers.

Experimenting with two Iowa soils, Brown and Gwinn (4) found that while applications of manure alone increased the availability of raw rock phosphate, the increase was much greater when sulphur was used in connection with the manure. They bring out the fact that there is a definite relationship existing between the sulphofying power of the soil and the production of available phosphorus.

Ames and Richmond (2) found that in an acid soil oxidation of sulphur proceeded vigorously, approximately 50 per cent of the sulphur being changed to the form of sulphate. In a basic soil the acidity resulting from sulphofication was partly neutralized, so that the solvent action on the rock phosphate was much less than occurred in the acid medium.

Since the inauguration of our work, Ames and Boltz (1) have published additional data concerning the effect of sulphur on soils and crops. These investigators found that both the nitrification of dried blood and the oxidation of sulphur in soil mixtures resulted in the liberation of potassium. They conclude that the liberation of the potassium was brought about by the salts formed rather than by the direct action of acidity on the insoluble potassium compounds.

PURPOSE AND PLAN OF THE INVESTIGATION

With the foregoing results in mind the present investigation was undertaken for the purpose of determining the effect of different composts upon the availability of the potassium of greensand. The investigation consisted of composting greensand with sulphur, soil, and manure in varying proportions, taking samples from time to time, extracting these samples with distilled water and analyzing the water extracts for the acidity, sulphate, and potassium contained.

Two series of composts were conducted, one series containing a greensand from Sewell, N. J., having a relatively high percentage of potassium, and the other a greensand from Crownsville, Md., having a rather low percentage of potassium. Each compost contained as a basis 1,500 gm. of greensand. The materials added were the same for each series and were as follows:

COMPOST NO.	MATERIALS ADDED TO GREENSAND.
1 and 8.....	Nothing.
2 and 9.....	500 gm. sulphur.
3 and 10.....	500 gm. sulphur; 500 gm. manure.
4 and 11.....	500 gm. sulphur; 250 gm. manure; 250 gm. soil.
5 and 12.....	500 gm. sulphur; 500 gm. soil.
6 and 13.....	500 gm. sulphur; 500 gm. soil; 0.02 per cent aluminum sulphate ($\text{Al}_2(\text{SO}_4)_3$) 0.18 H_2O ; 0.02 per cent ferrous sulphate (FeSO_4) 0.7 H_2O .
7 and 14.....	500 gm. sulphur; 250 gm. soil; 250 gm. manure; 10 gm. calcium carbonate (CaCO_3).

Commercial flowers of sulphur, partially rotted yard manure air-dried and ground fine, Collington sandy loam, and precipitated calcium carbonate were used. The aluminum and ferrous sulphates were added to composts 5 and 12 in order to determine whether these salts would exert a stimulating effect upon the rate and amount of sulphofication. McLean (11) found that, under certain conditions, these salts in combination exerted a marked stimulating action on sulphur oxidation processes when present in small amounts. He advocated the use of 0.4 pound per ton, or 0.02 per cent, of each for sulphur-floats composts. It was thought desirable to ascertain whether this effect would be obtained with sulphur-greensand composts.

METHODS OF PROCEDURE

The air-dry materials for each compost were weighed and thoroughly mixed. Similar smaller amounts of the same materials were mixed in the same proportions, from which the moisture-holding capacity of each compost was determined according to the Hilgard method (7, *p.* 209).

After being mixed, each compost was placed in a glazed pot, and water was added to one-half the determined water-holding capacity. The samples for the first analyses, showing the amounts of water-soluble

acidity, sulphate, and potassium at the start, were then taken, after which each compost was inoculated with the sulphofying organisms, and the aluminum and ferrous sulphates were added in solution to composts 6 and 13.¹

The period of composting was 23 weeks. Once each week the amount of water lost by evaporation was added, and the composts were removed from the pots and mixed, in order to provide thorough aeration.

The composts were kept in the greenhouse throughout the entire period and were covered at all times with a double thickness of white muslin to protect them from direct sunlight. The temperature of the greenhouse ranged from 50° to 100° F.

For the water extraction a 75-gm. sample was weighed from each compost, air dried, and 50 gm. of the air-dry material were shaken every half hour for 8 hours with 500 cc. of distilled water in a 1-liter Pyrex flask. After standing over night, the contents of the flasks were again shaken and filtered rapidly through folded No. 3 Whatman filter papers. The first 100 cc. of filtrate were poured back. The filtrates obtained were absolutely clear and free from sediment.

The acidity was determined by boiling aliquots of the water extract to expel carbon dioxid, cooling, and titrating with *N/10* sodium hydroxid, in terms of which the results are stated. Phenolphthalein was the indicator used. Titration was continued until all soluble iron, aluminum, and silica were precipitated and the clear solution retained the pink color for one minute.

Sulphur was determined by acidifying aliquots of the water extract with 2 cc. of concentrated hydrochloric acid and precipitating at the boiling point with barium chlorid. The results are expressed as sulphur trioxid (SO_3).

The potassium determinations were made gravimetrically by the platinic chlorid method from aliquots of the water extract, first eliminating the soluble organic matter, silicates, iron, aluminum, and phosphorus by evaporation with sulphuric acid, ignition, and subsequent precipitation. The determination for composts 1 and 8 throughout and the first three determinations for the other composts not containing manure were made colorimetrically because of the small amounts of potassium present.

Moisture determinations were made by heating separate 5-gm. portions of the air-dry compost for 15 hours at 105° C. All results reported in this paper are calculated to the moisture-free basis. No duplicate determinations were made, the idea being that one series of compost treatments would act as a control for the other in regard to the general trend of the reaction and that any serious error in analysis would

¹ Cultures containing sulphofying organisms were supplied by Dr. J. G. Lipman and Prof. A. W. Blair, of the New Jersey Experiment Station.

readily be shown and offset by the frequency with which the analyses were made.

The greensands, soil, and manure used were analyzed at the beginning of the investigation. The results are given in Table I. The potassium determinations were made by the official fusion method.

TABLE I.—Composition of materials used (dry basis)

Materials.	Moisture at 105° C.	Insoluble residue.	Ferric oxid (Fe ₂ O ₃), aluminum oxid (Al ₂ O ₃), phos- phorus pentoxid (P ₂ O ₅).	Calcium oxid (CaO).	Magne- sium oxid (MgO).	Potassium (K).
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
New Jersey greensand.....	5. 46	53. 12	31. 66	0. 16	1. 05	5. 88
Maryland greensand.....	1. 57	87. 83	8. 38	. 13	. 25	1. 42
Collington sandy loam.....	1. 20	89. 54	7. 54	. 18	. 22	. 83
Manure ^a	6. 30 49

^a Loss on ignition, 69.67 per cent.

Determinations made by the Veitch method showed that the New Jersey greensand required 4,200 pounds of calcium carbonate per 2,000,000 pounds, the Maryland greensand 3,400 pounds, and the Collington sandy loam 1,400 pounds.

The texture of the greensands and soil is shown in Table II, which gives the mechanical analyses of the materials used in the composts.

TABLE II.—Mechanical analyses of greensands and soil

Constants.	New Jersey greensand.	Maryland greensand.	Collington sandy loam.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Fine gravel.....	4. 94	1. 49	0. 53
Coarse sand.....	30. 26	1. 77	10. 14
Fine sand.....	45. 22	9. 15	26. 27
Very fine sand.....	15. 14	83. 65	43. 93
Silt and clay.....	4. 11	3. 86	18. 72

PRESENTATION AND DISCUSSION OF RESULTS

ACIDITY

In Table III is shown the acidity of the water extract from each compost as determined at the end of each 1-week period for the first 9 weeks and thereafter at the end of each 3 weeks for a total period of 23 weeks. The results are expressed in terms of *N/10* sodium hydroxid required to neutralize the acidity in the water extract from 10-gm. of compost on the dry basis.

TABLE III.—Accumulation of water-soluble acidity

Basis.	Com- post No	Materials added to 1,500 gm. greensand.	Cubic centimeters N/10 sodium hydroxid required to neutralize acidity of water extract from 10 gm. of compost (dry basis) after—														
			0 weeks.	1 week.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	7 weeks.	8 weeks.	9 weeks.	12 weeks.	15 weeks.	17 weeks.	20 weeks.	23 weeks.
New Jersey greensand..	1	None.....	.05	.075	.075	.075	.075	.075	.075	.05	.05	.075	.05	.05	.05	.05	.075
	2	Sulphur 500 gm.....	.05	.075	.10	.50	1.05	1.20	2.50	1.35	1.90	2.30	2.85	3.50	3.50	3.60	4.50
	3	Sulphur 500 gm.; manure 500 gm.	.05	.60	.70	3.50	8.05	17.20	36.75	59.15	151.35	156.50	146.25	126.20	151.00	157.85	159.20
	4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	.05	.25	.45	3.55	7.10	15.30	29.54	37.20	42.90	45.70	79.70	95.65	97.05	94.10	96.60
	5	Sulphur 500 gm.; soil 500 gm.....	.05	.075	.10	.65	1.35	1.90	2.55	3.10	3.70	4.80	7.30	12.15	18.95	25.10	35.45
	6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent Al ₂ (SO ₄) ₃ 0.18 H ₂ O; 0.02 per cent FeSO ₄ 0.7 H ₂ O.	.05	.075	.10	1.00	2.45	3.15	4.45	5.10	5.85	7.25	10.20	17.10	24.80	28.60	33.35
	7	Sulphur 500 gm.; soil 250 gm.; maure 250 gm.; CaCO ₃ 10 gm.	Alk.	.50	.60	1.50	38.25	65.00	62.10	59.80	61.25	64.40	97.65	105.40	104.95	102.80	101.35
Maryland greensand...	8	None.....	.05	.05	.05	.05	.075	.075	.10	.075	.05	.075	.05	.05	.05	.05	.05
	9	Sulphur 500 gm.....	.05	.05	.075	.35	.70	.95	1.30	1.30	1.90	2.05	2.80	3.75	4.35	4.05	5.15
	10	Sulphur 500 gm.; manure 500 gm.	.05	.70	.65	1.25	6.65	17.65	37.45	50.90	141.20	156.55	147.80	126.65	135.65	132.65	134.15
	11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	.05	.75	.70	2.90	7.30	18.05	30.40	37.80	42.95	73.20	112.85	111.10	114.85	110.55	116.85
	12	Sulphur 500 gm.; soil 500 gm.....	.05	.05	.15	.90	1.75	2.40	3.05	4.00	4.70	5.85	9.15	14.80	24.05	32.40	41.00
	13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent Al ₂ (SO ₄) ₃ 0.18 H ₂ O; 0.02 per cent FeSO ₄ 0.7 H ₂ O.	.05	.075	.10	.45	1.55	2.10	2.80	3.45	4.25	5.10	8.55	15.20	24.30	28.25	36.20
	14	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; CaCO ₃ 10 gm.	Alk.	.075	.75	1.30	6.55	10.90	26.70	57.50	103.90	104.00	103.35	107.40	108.75	108.85	112.15

Attention is called to the fact that, although both greensands showed a high lime requirement when tested by the Veitch method, neither of them gave evidence of more than a trace of acidity in the water extract. The addition of sulphur to the greensand in the proportion of 3 parts greensand to 1 part sulphur caused a gradual accumulation of water-soluble acidity, because of the slow oxidation of the sulphur. Composts 3 and 10, in which both sulphur and manure were mixed with the greensand, show a slight and gradual accumulation of water-soluble acidity up to the end of the fifth week, after which there is a very rapid rise for three weeks. For the remainder of the period the acidity fluctuates at a high and practically constant level. When one-half of the manure is replaced by an equal quantity of soil, as in composts 4 and 11, the acidity is greatly reduced, the maximum for the Maryland greensand being reached at the end of the 12-week period and for the New Jersey greensand after 15 weeks. When the manure was entirely replaced by soil, the acidity increased gradually throughout the entire period, as shown by composts 5 and 12; but the amount developed was only about one-third as much as when equal weights of soil and manure were used. This indicates rather strongly that in composts made up with a greensand deficient in calcium carbonate the rate of development and the amount of acidity depend very largely on the amount of organic matter present. A further comparison of composts 5 and 12 with 2 and 9 seems to substantiate this conclusion, in that the soil used contained a small amount of organic matter.

The acidity titrated did not, of course, at any time consist entirely of free sulphuric acid. As sulphofication progressed and the amounts of free sulphuric acid and sulphates increased, an increasing amount of acid silicates was obtained in the water extract and was precipitated upon titration with the alkali. Careful inspection of several titrations, made after the maximum acidity had been attained, seemed to indicate that from 45 to 55 per cent of the acidity titrated was due to free sulphuric acid, the remainder of the acidity being due to acid silicates and other acid salts.

Under the conditions of our experiment the addition of ferrous sulphate and aluminum sulphate when used at the rate recommended by McLean (11) for sulphur-floats composts has had no appreciable effect, as may be seen by a comparison of composts 6 and 13 with 5 and 12. The addition of 10 gm. of calcium carbonate to the sulphur-manure-soil compost had a marked stimulating effect, beginning about the third week in the New Jersey greensand compost and two weeks later in the Maryland greensand compost. In the former the stimulating action persisted up to the end of the experiment, while in the latter the effect of the calcium carbonate had entirely disappeared at the end of 12 weeks. A cause for this difference is found when the lime requirement of the New Jersey greensand is

compared with that of the Maryland greensand. As was previously mentioned, the lime requirement of the New Jersey material is 4,200 pounds of calcium carbonate, while for the Maryland greensand the requirement is only 3,400 pounds. The results recorded in Table III would appear to justify the conclusion that an initial acidity corresponding to a lime requirement of 3,400 pounds of calcium carbonate exerts a slightly depressing effect upon sulphofication, and that an acidity corresponding to a lime requirement of 4,200 pounds of calcium carbonate is less favorable. Ames and Boltz (1) found that calcium carbonate when added in excess of the lime requirements exercised a depressing effect upon the oxidation of sulphur in their soil-sulphur compost. When they reduced the application to half, the oxidation of sulphur increased but was less than when no carbonates were added.

SOLUBLE SULPHATES

A comparison of the results recorded in Table IV with those given in Table III shows that the accumulation of water-soluble sulphates parallels very closely the development of acidity.

It will be observed that the sulphur trioxid determinations fluctuate somewhat after having attained a maximum at the end of about 12 weeks. These fluctuations are probably due to variations in the moisture content and the temperature of the composts, since such variations are known to have an effect upon colloidal silicates, which in turn might exercise, through adsorption, an appreciable effect upon the soluble sulphur trioxid obtained in the water extraction. A calculation shows that at the end of our 23-week period, approximately 15 per cent of the total sulphur used in composts 3 and 10 had been oxidized, while for the composts in which one-half of the manure had been replaced by soil about 11 per cent of the total sulphur had been oxidized. These figures show that the amount of sulphur used was in excess of the amount necessary to secure the most economical results.

SOLUBLE POTASSIUM

The amount of water-soluble potassium in each compost at stated intervals is given in Table V.

A comparison of these figures with those given in Tables III and IV brings out the fact that with the increase in acidity and the accumulation of sulphur trioxid there is a corresponding increase in the amount of potassium in the water extract. The potassium, however, continues to increase for some weeks after the acidity and sulphur trioxid have reached a maximum. It seems necessary for a certain degree of acidity to be developed before any appreciable amount of potassium is made water soluble, the larger amounts of acidity and soluble sulphate breaking down the greensand more rapidly.

TABLE IV.—Accumulation of water-soluble sulphate

Basis.	Com- post No.	Materials added to 1,500 gm. greensand.	Milligrams water-soluble sulphur trioxid (SO ₃) in 10 gm. of compost (dry basis) after—														
			0 weeks.	1 week.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	7 weeks.	8 weeks.	9 weeks.	12 weeks.	15 weeks.	17 weeks.	20 weeks.	23 weeks.
New Jersey greensand.	1	None.....	0.68	0.93	0.98	1.36	1.79	1.58	1.69	1.87	1.94	1.51	1.73	1.91	1.61	1.73	1.76
	2	Sulphur 500 gm.....	1.07	1.57	1.89	8.45	11.94	13.59	14.62	15.82	17.63	18.91	22.99	25.33	26.93	27.47	31.92
	3	Sulphur 500 gm.; manure 500 gm....	4.68	22.70	34.67	68.59	93.85	133.60	206.60	298.98	660.99	714.09	698.99	638.28	740.72	811.80	812.09
	4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	2.57	7.72	17.75	47.03	64.44	100.41	156.44	187.27	213.53	224.87	382.81	408.39	473.47	475.97	485.74
	5	Sulphur 500 gm.; soil 500 gm.....	1.07	1.49	2.98	10.54	14.38	18.01	21.32	24.88	28.04	32.10	42.28	61.76	89.13	116.35	161.87
	6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent Al ₂ (SO ₄) ₃ 0.18 H ₂ O; 0.02 per cent FeSO ₄ 0.7 H ₂ O.	1.07	2.41	2.56	13.06	19.00	24.46	30.32	34.90	40.02	42.70	56.24	81.63	114.93	130.62	152.77
	7	Sulphur 500 gm.; soil 250 gm.; ma- nure 250 gm.; CaCO ₃ 10 gm.	3.50	23.25	47.61	59.66	213.11	320.30	313.29	309.21	317.44	330.69	492.98	543.58	549.24	546.50	535.55
Maryland greensand....	8	None.....	.42	.91	.84	1.69	1.47	1.65	1.79	1.82	2.43	2.00	2.56	2.46	2.14	2.31	2.14
	9	Sulphur 500 gm.....	.98	1.57	2.28	7.88	9.91	11.38	13.40	15.01	16.55	17.43	21.41	25.64	27.57	26.31	30.66
	10	Sulphur 500 gm.; manure 500 gm....	4.84	26.16	34.30	46.64	84.90	136.33	211.75	266.28	609.83	723.14	674.56	632.02	673.54	668.98	672.47
	11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	2.53	14.39	21.90	42.52	66.87	107.93	158.88	186.82	212.05	329.96	522.00	544.68	558.13	542.11	568.02
	12	Sulphur 500 gm.; soil 500 gm.....	.87	1.81	4.91	11.35	16.54	19.63	23.05	27.71	31.89	35.34	47.69	71.78	107.82	143.76	178.12
	13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent Al ₂ (SO ₄) ₃ 0.18 H ₂ O; 0.02 per cent FeSO ₄ 0.7 H ₂ O.	.80	2.09	3.10	8.47	14.62	17.52	22.45	25.14	29.24	32.30	44.18	72.85	109.26	125.09	158.08
	14	Sulphur 500 gm.; soil 250 gm.; ma- nure 250 gm.; CaCO ₃ 10 gm.	4.21	26.92	51.58	55.80	85.87	107.19	153.91	291.09	478.97	506.35	516.51	544.41	551.75	558.37	577.65

TABLE V.—Accumulation of water-soluble potassium

Basis.	Com- post No.	Material added to 1,500 gm. green- sand.	Milligrams water-soluble potassium in 10 gm. of compost (dry basis) after—														
			0 weeks.	1 week.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	7 weeks.	8 weeks.	9 weeks.	12 weeks. ^a	15 weeks.	17 weeks.	20 weeks.	23 weeks.
New Jersey greensand.	1	None.....	0.19	0.30	0.22	0.24	0.18	0.41	0.45	0.46	0.41	0.31	0.42	0.41	0.49	0.45
	2	Sulphur 500 gm.....	.24	.29	.45	.62	.98	.87	1.05	1.12	.98	1.20	1.20	1.30	1.17	1.43
	3	Sulphur 500 gm.; manure 500 gm..	2.99	5.75	7.43	9.87	8.05	11.57	10.11	11.68	14.77	17.64	40.04	48.46	62.56	64.11
	4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	1.47	2.70	3.56	5.55	4.68	5.85	5.99	6.10	8.98	8.18	25.91	31.25	30.10	32.77
	5	Sulphur 500 gm.; soil 500 gm.....	.32	.32	.48	.72	1.10	.87	.91	.96	1.07	1.02	1.30	2.08	3.18	3.36
	6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $\text{Al}_2(\text{SO}_4)_3$ 0.18 H_2O ;	.34	.34	.44	.75	1.05	.87	.97	1.17	1.16	1.27	1.34	2.25	3.10	3.36
	7	Sulphur 500 gm.; soil 250 gm.; ma- nure 250 gm.; CaCO_3 10 gm.	1.78	3.08	5.41	5.85	5.13	10.03	9.42	9.53	11.08	10.36	29.89	31.94	39.39	33.74
Maryland greensand....	8	None.....	.16	.21	.29	.27	.41	.48	.46	.52	.50	.3641	.46	.48	.48
	9	Sulphur 500 gm.....	.26	.24	.56	.52	.98	.72	.96	.99	.93	.9684	1.28	1.02	1.08
	10	Sulphur 500 gm.; manure 500 gm..	3.09	7.18	8.57	8.35	7.96	10.70	10.95	11.39	15.51	16.53	33.62	39.65	39.23	37.11
	11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.	2.54	3.48	4.15	5.33	4.37	5.80	6.04	6.21	9.25	8.36	23.74	28.02	26.75	28.16
	12	Sulphur 500 gm.; soil, 500 gm.....	.30	.30	.64	.65	1.03	.85	1.02	1.03	.88	1.1096	1.58	1.17	1.21
	13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $\text{Al}_2(\text{SO}_4)_3$ 0.18 H_2O ;	.28	.36	.56	.76	.84	.76	.84	.91	1.06	.9993	1.26	.95	.82
	14	Sulphur 500 gm.; soil 250 gm.; ma- nure 250 gm.; CaCO_3 10 gm.	2.30	4.22	6.27	5.86	4.77	6.90	5.71	6.35	11.97	10.22	23.46	25.26	25.33	26.53

^a No analyses made.

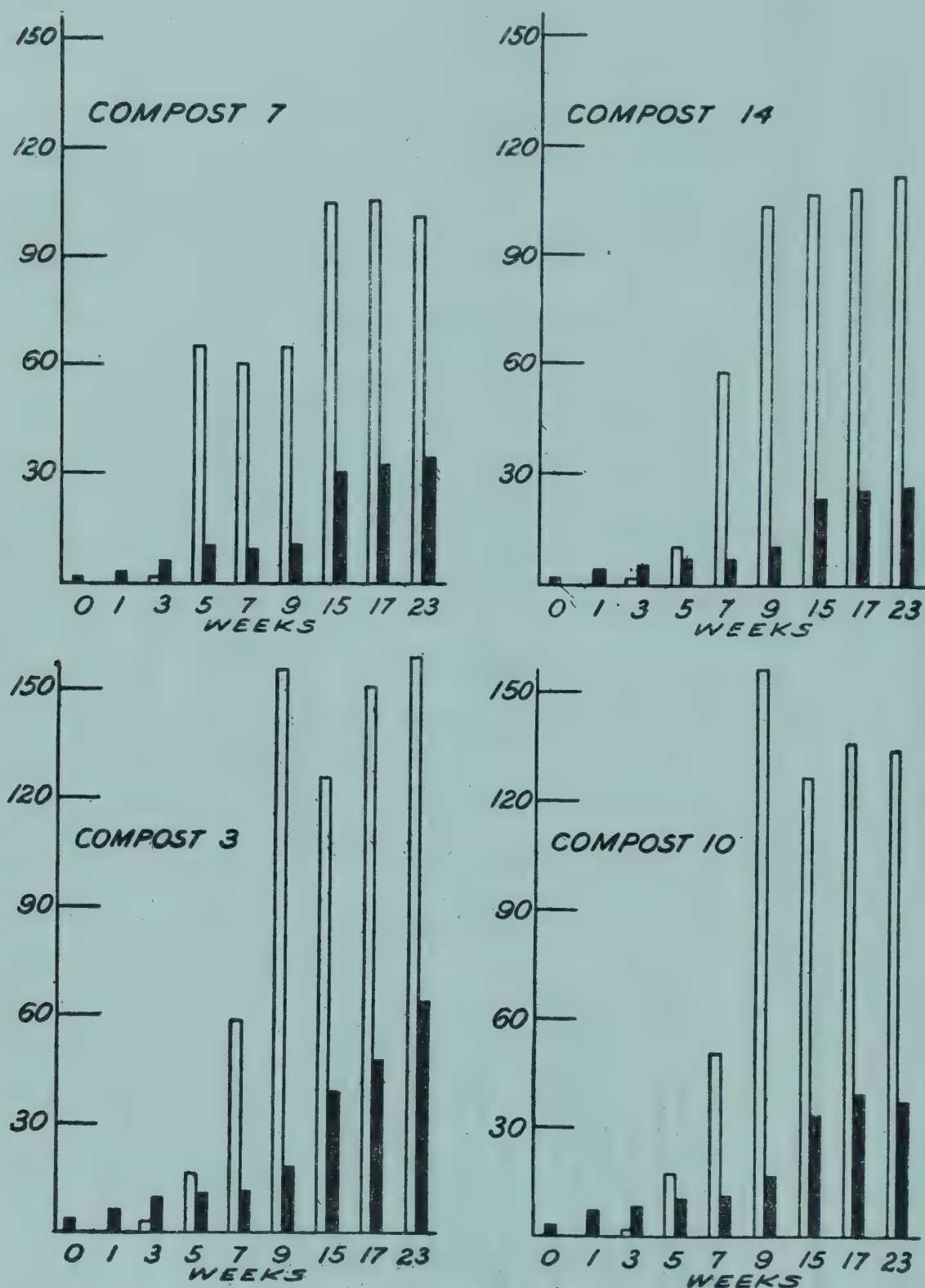


FIG. 1.—Diagrams showing relation of the water-soluble acidity to the water-soluble potassium at different time periods for different greensand composts. The open columns indicate the number of cubic centimeters of $N/10$ sodium hydroxid required to neutralize 10 gm. of compost on moisture-free basis, and the solid columns indicate the number of milligrams of water-soluble potassium obtained from 10 gm. of compost on moisture-free basis.

The diagrams of figure 1 give a graphic representation of the relation of the water-soluble acidity to the water-soluble potassium at different time periods for the greensand-sulphur-manure composts and for the greensand-sulphur-soil-manure composts to which were added 10 gm. of calcium carbonate.

A comparison of compost 3 with compost 7 and compost 10 with compost 14 brings out the fact that the replacement of one-half of the manure by soil has reduced the acidity and at the same time decreased the amount of potassium in the water extract. No. 7 and 14 show also the stimulation in acidity during the early weeks due to the addition of calcium carbonate.

The degree of acidity and the amount of sulphates and of potassium in the water extracts at the beginning of the period and at the end of 23 weeks for all the composts are shown in Table VI, which is a summary of Tables III, IV, and V.

TABLE VI.—Water-soluble acidity, sulphate, and potassium in water extract from 10 gm. of moisture-free compost at beginning and after 23 weeks of composting

Basis.	Com- post No.	Acidity (cc. N/10 sodium hydroxid required).		Sulfate (sulphur trioxid).		Potassium.	
		After 0 weeks.	After 23 weeks.	After 0 weeks.	After 23 weeks.	After 0 weeks.	After 23 weeks.
New Jersey greensand.	1	0. 05	0. 075	Mgm. 0. 68	Mgm. 1. 76	Mgm. 0. 19	Mgm. 0. 45
	2	. 05	4. 50	1. 07	31. 92	. 24	1. 43
	3	. 05	159. 20	4. 68	812. 09	2. 99	64. 11
	4	. 05	96. 60	2. 57	485. 74	1. 47	32. 77
	5	. 05	35. 45	1. 07	161. 87	. 32	3. 36
	6	. 05	33. 35	1. 07	152. 77	. 34	3. 36
	7	Alkaline	101. 35	3. 50	535. 55	1. 78	33. 74
	8	. 05	. 05	. 42	2. 14	. 16	. 48
	9	. 05	5. 15	. 98	30. 66	. 26	1. 08
	10	. 05	134. 15	4. 84	672. 47	3. 09	37. 11
Maryland greensand.	11	. 05	116. 85	2. 53	568. 02	2. 54	28. 16
	12	. 05	41. 00	. 87	178. 12	. 30	1. 21
	13	. 05	36. 20	. 80	158. 08	. 28	. 82
	14	Alkaline	112. 15	4. 21	577. 65	2. 30	26. 53

Attention is called to the fact that the potassium liberated from the New Jersey greensand is much greater than that recovered from the Maryland greensand. This is to be expected, since the former had an initial potassium content of 5.88 per cent, while the latter contained only 1.42 per cent of potassium, as shown in Table I. It will be seen that the largest amount of potassium was extracted from compost 3, containing the New Jersey greensand, and the second largest amount from compost 10, which is the corresponding mixture made with Maryland greensand. The fact that both of these composts have twice the amount of manure contained in No. 4, 7, 11, and 14 would indicate that

comparatively large amounts of organic matter favor sulphofication and the liberation of potassium under the conditions of this experiment. These results are not in accord with those reported by McLean (11), who, working with sulphur-floats-soil composts, came to the conclusion that a compost is more efficient in the producing of available phosphorus in the absence of large amounts of organic material.

In Table VII the total potassium present in each compost, the water-soluble potassium at the start, and the maximum water-soluble potassium present at any one time during the period of 23 weeks are computed on the basis of the initial weights of the composts.

TABLE VII.—*Total potassium made water-soluble (dry basis)*

Com- post No.	Material added to 1,500 gm. greensand.	Total number grams potas- sium in compost.	Water- soluble potas- sium at start (percent- age of total).	Maximum water- soluble potassium present.	
				Gm.	Percent- age of total.
1	None.....	83.38	0.037	0.070	0.084
2	Sulphur 500 gm.....	83.38	.055	.275	.330
3	Sulphur 500 gm.; manure 500 gm.....	85.68	.832	15.28	17.83
4	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.....	86.58	.408	7.87	9.10
5	Sulphur 500 gm.; soil 500 gm.....	87.48	.088	.812	.928
6	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $\text{Al}_2(\text{SO}_4)_3$ 0.18 H_2O ; 0.02 per cent FeSO_4 0.7 H_2O	87.48	.094	.812	.928
7	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; CaCO_3 10 gm.....	86.58	.494	9.46	10.93
8	None.....	20.97	.112	.070	.333
9	Sulphur 500 gm.....	20.97	.243	.251	1.20
10	Sulphur 500 gm.; manure 500 gm.....	23.27	3.22	9.62	41.34
11	Sulphur 500 gm.; manure 250 gm.; soil 250 gm.....	24.17	2.57	6.88	28.50
12	Sulphur 500 gm.; soil 500 gm.....	25.07	.295	.389	1.55
13	Sulphur 500 gm.; soil 500 gm.; 0.02 per cent $\text{Al}_2(\text{SO}_4)_3$ 0.18 H_2O ; 0.02 per cent FeSO_4 0.7 H_2O	25.07	.275	.310	1.24
14	Sulphur 500 gm.; soil 250 gm.; manure 250 gm.; CaCO_3 10 gm.....	24.17	2.33	6.49	26.85

Reference to the last two columns of Table VII will show that, while the actual amount of soluble potassium which formed in the composts containing the Maryland greensand was much smaller than that which formed in the composts containing the New Jersey greensand, the percentage of the total potassium made water-soluble in the former was much greater than in the latter. One of the causes for this difference is to be found in Table II, which shows the mechanical analyses of the two greensands. The individual particles are much smaller in the Maryland than in the New Jersey greensand, thus exposing a much greater surface to the solvent action of the acids. Also, the glauconite particles of the

former were softer than those of the latter and seemed to be more soluble, as is shown by composts 1 and 8 in Table V. These figures show that although the New Jersey greensand contains more than four times as much potassium as the Maryland greensand, the amount of water-soluble potassium is the same.

In considering Table VII it is pertinent to ask to what extent the manure has contributed to the total amount of potassium recovered in the water extract. To answer this question Table VIII has been prepared upon the assumption that all the potassium in the manure was made soluble and was recovered in the water extract.

TABLE VIII.—Relation of potassium content of the manure to the water-soluble potassium obtained

Compost No.	Total soluble potassium obtained from compost.	Total potassium in manure.	Maximum amount of potassium from manure. ^a
	Gm.	Gm.	Per cent.
3	15.28	2.30	15.05
4	7.87	1.15	14.62
7	9.46	1.15	12.16
10	9.62	2.30	23.91
11	6.88	1.15	16.72
14	6.49	1.15	17.72

^a The percentages in this column are based on the assumption that all the potassium in the manure was made water-soluble.

From the last column of Table VIII it will be seen that even on this basis it is possible in only one case to account for more than 17 per cent of the potassium as coming from the manure. It is evident, therefore, that from 80 to 90 per cent of the potassium found in the water extract must have come from the greensand or from the soil and greensand.

Referring again to the manure composts in Table VII, it will be seen that the total amount of potassium recovered by water extracts from these composts varies from 9.1 per cent to as much as 41.3 per cent of the total initial amount present.

It is important to consider the relation between the oxidation of sulphur and the liberation of potassium. This relation is a converging ratio, which was rather wide during the period of greatest oxidation of sulphur and diminished rapidly as the potassium was released. While it was not expected that this ratio would be resolved to a constant figure for all of the composts, because of the different materials used, in each series the composts containing manure do show a rather uniform relation between these processes. On the basis of the initial weights of the composts, Table IX shows the maximum number of grams of sulphur oxidized and of water-soluble potassium obtained, and their ratio, as determined from the water extracts.

TABLE IX.—*Relation between number of grams of sulphur oxidized and number of grams of potassium made water-soluble*

Compost No.	Sulphur oxidized.	Potassium made water-soluble.	Ratio of grams sulphur oxidized to grams water-soluble potassium.
	<i>Gm.</i>	<i>Gm.</i>	
3	77.43	15.28	5.07:1
4	46.65	7.87	5.92:1
7	52.79	9.46	5.58:1
10	70.15	9.62	7.29:1
11	55.51	6.88	8.07:1
14	56.52	6.49	8.70:1

In the New Jersey greensand composts, approximately $5\frac{1}{2}$ gm. of sulphur were oxidized for each gram of potassium made water soluble. For the Maryland greensand composts, the ratio is approximately 8 to 1. The ratio varies with the materials used, the high-potassium greensand having a lower ratio than the low-potassium greensand, and the composts containing 20 per cent manure having a lower ratio than those containing 10 per cent manure. For the composts in which soil was substituted for all the manure the figures are not shown, but the ratio is much wider, the amount of sulphur oxidized not being sufficient to make water soluble any large amount of potassium.

The results of this investigation would indicate that the composting of greensand, or of soil rich in potassium, with sulphur and manure may prove to be a practical and efficient method for obtaining available potassium from comparatively insoluble materials.

SUMMARY

Two greensands, one containing 5.88 per cent of potassium and the other 1.42 per cent, were used in studying the effect of sulphofication upon the solubility of the potassium. The outstanding results of the investigation are summarized in the following paragraphs.

(1) In composts consisting of greensand, manure, and soil in different proportions, an appreciable amount of the potassium of the greensand was made water-soluble through sulphofication.

(2) The composts containing the largest proportion of manure developed the highest degree of acidity, oxidized the greatest amount of sulphur, and produced the largest quantity of water-soluble potassium.

(3) The composts in which soil was substituted for a part of the manure developed less acidity, oxidized less sulphur, and produced a smaller amount of soluble potassium.

(4) When all the manure was replaced by soil, the rate of sulphofication was so slow that at the end of 23 weeks only a very small amount of acidity had developed and very little potassium had been made soluble.

(5) When no organic matter was added, the amounts of acidity and soluble sulphates were no greater than might be accounted for by the natural oxidation of the sulphur.

(6) The addition of small amounts of ferrous and aluminum sulphates failed to stimulate sulphofication.

(7) Calcium carbonate added to the sulphur-manure-soil compost produced a stimulating effect during the early part of the period but failed to increase the acidity, soluble sulphates, or potassium above the maximum reached by the corresponding compost in which no calcium carbonate was used.

(8) More water-soluble potassium was formed in the composts containing the high-potassium greensand, but a larger percentage of the total potassium present was liberated in the composts containing the low-potassium greensand.

(9) In the composts containing manure, the total amounts of potassium recovered in the water extracts varied from 9.1 per cent to a maximum of 41.3 per cent of the total initial amount present.

(10) Our results indicate that the composting of greensand, or of soil rich in potassium, with sulphur and manure may prove to be a practical and efficient method for obtaining available potassium from comparatively insoluble materials.

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RUST IN SEED WHEAT AND ITS RELATION TO SEEDLING INFECTION¹

By CHARLES W. HUNGERFORD

Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The fact that the mycelium of rust fungi in some cases may enter the seed and seed parts of various plants and produce spore bodies there has been known for many years and has been referred to by various writers. Differences of opinion have existed, however, as to the importance of this phenomenon in the dissemination of the rust concerned. Aside from the occurrence of rust in and upon these plant organs, other facts have seemed to indicate that rust might be transmitted by means of seed. A number of cases are on record where the uredinial and telial stages of various rusts have suddenly appeared in regions where the aecial host was unknown. Lagerheim (17)² found *Puccinia coronata* Cda. on oats in Ecuador, and since no species of *Rhamnus* known to bear the aecia of this rust occur there he concluded that the rust was probably introduced by means of oats brought from Europe. He also reported stemrust doing great damage in Ecuador, although barberry bushes were not present there. According to McAlpine (18, p. 60), *P. graminis* is common in Australia, while only a very few hedges of barberry exist and the aecial stage of this rust has never been found occurring naturally upon that continent. Bolley and Pritchard (5, p. 647) quote McAlpine as saying that he is convinced that certain grass seeds secured by him from the United States Department of Agriculture introduced certain rusts into Australia. Among these he named *P. coronata* Cda. on the grass *Beckmannia erucaeformis* and *P. montanensis* Ell. on wild rye (*Elymus canadensis*). Numerous other similar instances could be cited.

The widespread occurrence of rust epidemics has not been satisfactorily explained, to some pathologists at least, by our present knowledge of the overwintering of the uredinial stage or by our present knowledge of the importance of infection of wheat by aeciospores from the barberry. These conditions have caused a number of writers to attempt to explain sporadic attacks of rust by a theory of seed transmission. The idea is

¹ The investigations reported in this paper were carried on at Madison, Wis., under the direction of the Office of Cereal Investigations, United States Department of Agriculture, Washington, D. C. The writer wishes especially to thank Dr. L. R. Jones and Dr. A. G. Johnson, of the Department of Plant Pathology of the University of Wisconsin, and Dr. H. B. Humphrey, of the Office of Cereal Investigations, United States Department of Agriculture, for helpful suggestions and criticisms during the progress of the work and in the preparation of the manuscript.

² Reference is made by number (*italic*) to "Literature cited," p. 275-277.

not new that certain fungous parasites may exist in the vegetative state in the seeds of their hosts and be thus transmitted from one generation to another. Even before this was established for certain of the cereal smuts, various workers had endeavored to show this condition for the cereal rusts. The discovery that certain smuts were systemic in their infection gave impetus to further research along this line.

The purpose of the investigations reported here was to determine whether or not *Puccinia graminis tritici* Erkiss. and Henn. can be transmitted to the seedling by being carried over with the seed grain.

OCCURRENCE OF RUST IN SEEDS AND SEED PARTS OF VARIOUS PLANTS

The earliest report that the writer has been able to find that stemrust may attack the seed and seed parts of grain was made by W. G. Smith in 1885 (23). He found telia of *Puccinia graminis* in the pericarp of oat kernels and figured teliospores within the oat grains lying inside the aleurone layer and between that and the endosperm. His drawings and notes, however, leave much to be desired. In 1886 (24) the same author figured aecia embedded in the fruits of the barberry. Maddox (18, p. 20) noted rust infection upon—

the young half-grown grain . . . before it had started to go out of the milk stage.

He does not state to which rust he refers. Pritchard (22, p. 151) in 1911 figured stemrust upon wheat kernels and stated that telia and fragments of mycelium were found in abundance in the pericarp of wheat kernels and that seed infection occurs very frequently even in rust-free years. Other reports have been made of *P. graminis* upon the caryopses of wheat, oats, barley, and various grasses, and the writer has observed this condition upon all of the above-mentioned hosts.

Puccinia glumarum (Schm.) Erikss. and Henn. is also known to occur commonly upon the caryopses of wild and cultivated Gramineae. Beauverie (1, 2) has recently reported at length upon this phenomenon and states that if the seed is hulled the sori are produced upon the interior of the glumule, while if the seed is naked they are formed in the pericarp. He found this rust more or less abundant in the caryopses of *Triticum vulgare*, *Hordeum vulgare*, *Brachypodium pinnatum*, *Agropyron caninum*, and *Bromus mollis*. He also reports finding *P. simplex* on barley kernels and *P. coronata agropyri*¹ on *Agropyron repens*. Blaringhem (3, p. 86) found somewhat the same conditions reported by Beauverie. Eriksson and Henning (10, p. 199, pl. 7, 9) fully describe and give excellent figures of whole kernels and cross sections of kernels infected with *P. glumarum*.² Various other rusts have been reported as occurring upon seeds and seed parts of various plants. Carleton (6, p. 28-29) has reported the occur-

¹ It is not clear what rust is referred to by this name.

² These authors cite several former observations of *P. glumarum* upon kernels of wheat, the earliest of which was by Schmidt (10, p. 454) in 1819.

rence of Euphorbia rust (*Uromyces euphorbiae* C. and P.) upon seeds of *Euphorbia dentata*. Various writers have noted *P. malvacearum* Mont. on hollyhock seeds. Other examples of a similar nature could be given. The discussion of the practical importance of this occurrence in relation to subsequent infection of seedlings will be taken up in a later paragraph.

ABUNDANCE OF KERNEL INFECTION IN WHEAT

In order to learn to what extent seed wheat may become infected with *Puccinia graminis tritici* a large number of wheat samples were examined by the writer. These samples were secured from various sources and from the crops of the two years 1915 and 1916. During the fall and winter of 1915-16 samples of wheat were secured from various points in North and South Dakota, from western Minnesota, from grain commission firms in Minneapolis, and from wheat grown in the rust nursery at the University Farm, St. Paul, Minn. In all, several hundred samples of wheat were examined, all of which came from fields known to be badly rusted or from localities where it was known that rust epidemics had occurred. During the fall of 1916 a large number of samples of wheat were obtained from the same regions as in the previous year. In those regions there occurred that year an unusually severe rust epidemic. It would seem, therefore, that under these conditions there would be as large an amount of seed infection as ever occurs.

It was found at once that the task of determining the percentage of infection was not so easy as it at first appeared. In some cases the kernels were found to be but slightly infected, having only one sorus on the hilum, or germ end. In such cases it was impossible to see that these were infected at all except by means of examination under considerable magnification. In other cases the general appearance of the kernels seemed to indicate to the unaided eye that there was rust infection, but upon examination under the microscope no rust was found. Indications were that such discolorations were caused by some other agency. *Alternaria* and *Helminthosporium* species were often found to be associated. In general, it was found impossible to tell in every case whether or not a kernel was infected by rust except by microscopic examination. However, in many cases, especially after some experience, many of the rust-infected kernels could be easily detected by the unaided eye.

The large majority of the rust-infected kernels, when mature, were found to bear only telia,¹ which appeared as glistening black specks on the hilum, or the germ end, or a short distance down the groove of the kernel. Sometimes sori were noted a short distance from the hilum with no surface connections between these and the ones at the hilum (see Pl. 39, B). Upon sectioning similar kernels, however, the mycelial connections were found. If the hilar end of an infected kernel is scraped with a sharp knife or scalpel, teliospores in abundance can be secured.

¹ Uredinia were noted on immature kernels at various times.

In order to learn the percentage of infection and also to be absolutely certain that all seed used in experimental work was infected with rust, the following method of selecting rusted kernels was employed. The samples of wheat to be examined were spread out in a shallow dish where the light was good, and the discolored kernels were taken out one by one by means of small forceps. A common 5-inch reading glass usually was used to facilitate making the selections. These discolored kernels were then placed one by one under a low-power binocular microscope where it could be easily determined at a glance if any rust sori occurred on their germ ends. As will be shown later, some infected kernels may have been missed, for sometimes the sori on the germ ends are broken off with the flowering glumes in thrashing.

Bolley and Pritchard (5, *p.* 646) state that—

in some samples of wheat in the rust-infected crop of 1904 as high as 30 per cent of all grains harvested showed such rust infection.

Pritchard (22, *p.* 153) also states that in 1910, a rust-free year, wheat from elevators at Brookings, S. Dak., showed some rusted kernels in every sample and many in some varieties, especially Bluestem. The writer's observations do not agree with this. In all the hundreds of samples examined the largest percentage of kernels found in any one sample showing rust sori was only about 1 per cent of the total. Many samples were examined in which no infected kernels could be found. In fact, even in 1916, a very bad rust year, the varieties having kernel infection were the exception rather than the rule. Moreover, varieties of the durum wheat were the ones which most often were found to be infected. This was the case in both years and seems to be consistently so. One sample of mixed wheat from Reeder, N. Dak., collected in 1916, contained about 1 per cent of infected kernels. Although the sample contained Marquis, durum, and Bluestem in the mixture, only durum kernels were found infected. This has been found to be the case in many mixed samples examined. Only in a few cases have any number of infected kernels of other varieties been found. This may be due to the fact that the spike of durum wheat is so compact that it dries very slowly after rains or heavy dews and these moist conditions favor infection by rust. It is a well-known fact that durum varieties are very susceptible to *Fusarium* scab, possibly for the same reason.

To illustrate this point the following observation is of interest. The writer noted in 1916 at Dickinson, N. Dak., that all the durum wheats were more or less badly rusted on the heads. (See Pl. 38.) This was especially true of the Kubanka strain known as selection No. 8, C. I. No. 4063 (Pl. 38). A large number of heads of this variety were collected which were literally covered with stemrust sori (Pl. 39, A). Mr. Ralph Smith, of the Office of Cereal Investigations, stationed at Dickinson, kindly furnished the writer some of the seed of this variety when the plots were thrashed. This seed was all examined carefully, and it was

found that only about one kernel in a thousand showed any evidence of rust infection.

METHOD OF KERNEL INFECTION

There are two possible methods by which kernel infection takes place. First, the kernel itself may become infected by urediniospores lodging upon its surface under the glumes; or, second, the infection may spread from sori produced upon the inclosing glumes, upon the rachis or the rachilla. Since there probably are no stomatal openings upon the kernel itself and since uredinial infection takes place only through the stomata, the first possibility seems to be eliminated. Cobb (7) reports finding urediniospores of stemrust in abundance in the brush of the kernel of a large number of varieties of wheat, even after the wheat was thoroughly cleaned. He also reports finding stomata near the brush end and concludes that infection of the kernel may take place at this point. He found sori common on wheat kernels but does not say anything with regard to their location.

The writer has never found sori of stemrust produced near the brush end of wheat kernels nor has he been able to find stomata upon wheat kernels at any time in their development. As previously stated the writer has found rust sori on wheat kernels at or near the germ end. This would indicate that infection takes place by the spread of rust mycelium to the caryopsis from infection which had previously taken place at the base of the glumes or on the rachilla. Indeed, our experiments have confirmed this. When kernels were examined in the wheat head and were found to be infected, it was found that one or more of the flowering glumes always bore sori; and frequently several sori on the rachis, rachilla, and glumes were found to be confluent and extending over to the base of the kernel. The tissue of the hilar region of the kernel is similar in its structure to leaf tissue, and therefore infection of this region might be expected. In samples of thrashed grain kernels with adhering pieces of glumes often had rust sori extending from the base of the glume to the kernel itself. The glumes seemed to be held thus by the fungus (Pl. 39, B).

That infection may spread from the glumes to the kernel hilum was shown as a result of artificial inoculation experiments. These were carried out as follows. Artificial inoculations of wheat heads with urediniospores of stemrust were made in the greenhouse during the winter of 1915-16. The first set of inoculations was made when the kernels were less than half grown. Urediniospores were dusted in abundance inside the glumes, and the heads were sprayed with distilled water, inclosed in large test tubes, and kept for two days. Wet cotton was kept in the bottoms of the tubes and the mouths were plugged with cotton, thus giving the conditions necessary for infection. The first attempt was a failure, either because too many spores were used or

because the kernels were not developed far enough to survive the invasion of the parasite, and the infection was so great that none of the kernels developed. The glumes and rachis at the base of the spikelet in each case were covered with sori 10 days after inoculation and the inner surfaces of the glumes were filled with urediniospores.

These results appear to confirm Johnson's (15) observations regarding the effect of rust infection upon floret sterility in wheat. He found floret sterility increased 20.03 per cent when wheat heads were sprayed with a water suspension of a mixture of urediniospores of *Puccinia graminis* and *P. triticina*. His conclusions were that when the rust attacks the ovary early enough it prevents its development, and other semiparasitic fungi complete the process of destruction, while if it attacks the embryo after it is fertilized and has begun to enlarge, a rusted kernel results. Table I shows the outcome of a second set of inoculations. Kubanka wheat (C. I. No. 1440) was used for these experiments. The glumes were opened, and a very few spores were placed at the base of the inside of the glumes with a fine platinum needle. The heads were then sprayed with distilled water and inclosed in a test tube as before. Every spikelet in each head, with the exception of the smallest ones at the tip, was thus inoculated.

TABLE I.—Results of artificial inoculation of wheat ovaries at different stages of development

Host No.	Condition of ovaries.	Date of inoculation.	Number of heads inoculated.	Date thrashed.	Number of infected kernels.	Number of healthy kernels.
5	Ovaries two-thirds grown.	Nov. 15, 1915	2	Jan. 11, 1916	3	8
6do.....	Nov. 18, 1915	1do.....	2	1
7	Ovaries size of pinhead.do.....	1do.....	0	0
8do.....do.....	1do.....	0	1
9	Ovaries somewhat larger than above.do.....	10do.....	0	2
10	Kernels two-thirds grown.	Nov. 15, 1915	4do.....	3	5
11do.....	Dec. 5, 1915	3do.....	15	8

It will be noted from Table I that in no case was kernel infection obtained when inoculations were made while the ovary was very small. On the other hand, when the inoculations were delayed until the kernels had attained about two-thirds of their normal size at maturity, the kernels were able to continue development, and a high percentage of rusted ones resulted. It would seem, therefore, that the amount of kernel infection each year does not depend alone upon the amount of rust occurring upon the heads of the wheat but also upon the time when this infection takes place and whether the kernels are at the right stage of development to become infected. The weather conditions where the kernels are at the right stage of development are also a very important factor.

EFFECT OF KERNEL INFECTION UPON GERMINATION

Large numbers of rust-infected wheat kernels were germinated and grown to various stages of development for the purpose of making histological studies. Parallel series of unrusted kernels from the same seed lot were germinated and grown for comparison. In these series it was noted that the rusted and unrusted seed gave practically identical percentages of germination.

RUST TRANSMISSION WITH SEED GRAIN

HISTORICAL DISCUSSION

From the vast amount of work which has been done upon this problem it is possible to separate three main theories. Briefly stated, these theories are as follows: (1) Mycoplasma theory of Eriksson; (2) dormant mycelium in the seed carrying infection to the seedling; and (3) seed-borne spores causing infection of the seedling.

MYCOPLASM THEORY OF ERIKSSON

Eriksson (9) in 1897 announced his well-known mycoplasma theory. He states that in the summer of 1893, upon microscopical examinations of sections of very young sori of yellow-rust (*Puccinia glumarum*) upon wheat leaves, he found adjacent to these sori, besides the usual cell elements, peculiar, elongated, mostly faintly curved, plasmatic corpuscles. He concluded (*p.* 193, translation) that—

these plasma corpuscles, at first freely swimming in the protoplasm, constitute a phase of the fungus, the primary phase, wherein the fungus by its independent appearance makes itself visible. The fungus has for weeks, months, possibly even years, previously led a latent existence in an invisible form and alongside the protoplasm of the host plant, forming a kind of mycoplasma-symbiosis between host and parasite.

Although Eriksson describes this mycoplasma in detail and figures it in various stages of development, very few later writers have accepted his evidence as being in any way conclusive. While it is not the present purpose to give a detailed criticism of the theory, yet, in the judgment of the writer, it seems that Eriksson's experimental evidence does not establish his contention in regard to the existence of the so-called mycoplasma. Nothing similar has been encountered in any of the hundreds of sections which the writer has made. More will be said later of this experimental evidence upon which Eriksson based his conclusions. Ward (25, *p.* 353) sums the matter up very well when he states that Eriksson merely—

inverts all the stages of the fungous attack on the cell, and supposes the last stage to be the first and that this error and misrepresentation of the microscopic appearance account for the whole wearisome persistence in an inherently improbable hypothesis.

Detailed criticisms of Eriksson's theory are given by Bolley (4), Zukal (26), Ward (25), and Masee (20). Others could be added to this list,

but it is sufficient to say that no pathologist of note has for any length of time accepted this explanation of rust dissemination.

DORMANT MYCELIUM THEORY

There has been more support, and probably more ground for support, for the theory that the mycelium of rusts may live over in the seed or seed parts of the plant in a dormant state and then infect the young seedlings at the time of germination. A number of writers have suggested this possibility, among whom W. G. Smith (24) was probably the first. He says:

If apparently healthy leaves of corn are taken, and apparently healthy leaves of Barberry, and these leaves are microscopically examined, fungus mycelium will be commonly found inside the leaves. Neither is the mycelium confined to the leaves, for it invades the seeds of both plants, and these seeds are frequently planted with the mycelium in their tissues. A diseased progeny is the result.

Zukal (26), in 1899, published observations which seemed to indicate to him that rust was transmitted by mycelium in seed grain. He concluded that rust mycelium might live over in the wheat kernels because the rust appeared so early on the young seedlings. He found septate mycelium at the base of the sheath, in the culms, and at the nodes in the parenchyma cells just under the epidermis. He concluded that the mycelium lived over in the seed and in the spring grew through the scutellum into the embryo and developed with the plant.

Pritchard (22, p. 152), in 1911, found mycelium in the roots, in both central cylinder and epidermis, in the stem, and between the leaf sheaths in plants grown from rusted wheat kernels. This mycelium resembled rust mycelium which he found at the base of the sori upon the germ end of the kernel of wheat from which the plants were grown. He states that the mycelium was abundant in the young stem, filling the intercellular spaces and freely penetrating cell walls as well. More will be said later in regard to Pritchard's work.

SEED-BORNE SPORES THEORY

Massee (20) secured evidence which seemed to indicate to him that seed-borne urediniospores or urediniospores in the soil might cause infection of young wheat plants. More recently Blaringhem (3) and Beauverie (1) have published extensive observations which they have made. They conclude that *Puccinia glumarum* may be transmitted by urediniospores borne in the pericarp of the seed. As stated above, they found uredinia in abundance in the pericarp of various grains and grasses and concluded that these spores, so protected, may retain their viability until the germination of the seed, when they become free from the sori through the rupturing of the pericarp and may infect the young plant at this time. Their conclusions, in the writer's judgment, are based upon insufficient experimental evidence, and, although the theory is interesting in itself, certainly it should be supported by more careful experiments.

EXPERIMENTS OF VARIOUS WORKERS

A number of workers have grown plants from rusted seeds of various kinds under various degrees of isolation. The results of these experiments are rather variable. The writer has assembled the results and the methods used in several of these experiments in Table II, which includes the experiments of nine men conducted at different times in different countries. None of these writers claimed to have secured normal conditions for the growth of the host plants, and in no case was any record taken of the atmospheric conditions inside the devices used to secure isolation.

TABLE II.—Summary of results obtained by other investigators in experiments on seed transmission of rusts

Experimenter.	Year.	Place of experiment.	Rust involved.	Means of isolation.	Kind of seed used.	Results.
Eriksson (9)...	1892-1898	Sweden...	<i>P. glumarum</i> , <i>P. graminis</i> .	Ventilated glass frames.	Barley, wheat	Few positive.
Klebahn (16)...	1899	Germany...	<i>P. graminis</i> , <i>P. glumarum</i> .	Glass cages.....do.....	Uncertain.
Zukal (26).....	1898	Austria....	<i>P. glumarum</i>	Isolated garden..	Wheat.....	Negative.
Linhart ^a	1898do.....do.....	Glass inclosures.do.....	Do.
Hayman (12)...	1903-1907	India.....	<i>P. glumarum</i> , <i>P. triticea</i> .	Glass cages.....do.....	Uncertain.
Bolley (4).....	1905	North Dakota.	<i>P. graminis</i>do.....do.....	Negative.
Massee (20)....	1894	England...	<i>P. glumarum</i>	Bell jars.....do.....	Positive.
Nowikoff ^b		Russia.....	<i>P. coronata</i> , <i>P. glumarum</i> .	Isolated cages...	Oats, barley..	Negative.
Jaczewski (14).	1902-1906do.....do.....	Glass cages.....	Oats, rye.....	Do.

^a Reference is made to Linhart's work by Zukal (26); original work not published.

^b Referred to by Jaczewski (14); original not seen.

Eriksson carried on experiments for seven years and secured only a very few infections upon plants grown inside his "isolation frame." This frame was made of glass with wooden corner posts and an iron roof. Ventilation was secured by drawing air through a cotton filter. At best a cotton filter is not very satisfactory, and it is to be noted that Eriksson secured his positive results after the cages had been used three or four years. Grove (11, p. 45-47) makes an interesting comment upon Eriksson's work. He says (p. 45)—

on some of his "protected" plants aphides also made their appearance, yet this does not seem to have suggested to him [Eriksson] that the *zooplasm* of the aphides must also have been latent in the seed. If the aphides got in, so would fungus spores, since it has been proved that uredospores are carried by them and other insects.

Klebahn repeated Eriksson's experiments and found one plant infected with *Puccinia graminis* in his glass cages. He explains this (16) by the fact that this infection did not appear until a few days after he had been working with *P. graminis* near this cage. The time which had elapsed was about the normal incubation period for this rust. It seems very likely, therefore, that the one infection noted originated from spores accidentally introduced.

Hayman (12) repeated his experiments for five years and grew 195 plants to maturity. The conditions inside his cages were abnormal at all times, although an effort was made to control conditions by means of a blacksmith bellows and cotton filters. Two pustules of rust appeared in the fifth year, but the author himself was not satisfied with this result as is evidenced by the fact that he states that the tar used to coat the inside of the cages had oozed through the cracks in the cage in which the plant was found to be infected.

Massee, the only other worker who secured positive results, used bell jars placed upon cotton wool with a cotton plug in the opening at the top. He sowed wheat inside these jars, which was known to be shriveled by *Puccinia glumarum*, and as controls he sowed plump seed of the same variety. Sixty per cent of the infected seed germinated, and when the plants were 3 inches high rust appeared in each pot. When the plants were 5 inches high 26 per cent of them were rusted. Of the plump seed sown under the same conditions 96 per cent germinated, and all remained perfectly free from rust. These results are striking, and the problem with this rust is highly deserving of further investigation.

Pritchard (21) grew 60 wheat plants from rusted seed in glass cages in the open and later repeated the experiment in the greenhouse. No rust appeared on any of the plants. He states that the plants headed and blossomed but no kernels developed because the temperature and moisture conditions were abnormal. He also refers to an experiment where wheat sown at different dates was inoculated with both aeciospores and urediniospores of stemrust. Rust did not appear abundantly, however, until the wheat began to head, when each sowing became thoroughly rusted. He states that it is possible to attribute this peculiar behavior to infection through the seed with a long subsequent incubation period in the growing plant. It seems to the writer that this conclusion is entirely unwarranted, since it is well known that infection with stemrust is much more easily obtained and more noticeable during the heading period of the plant when stemrust does such great damage by attacking the neck of the stalk. This is a period of rapid growth of the plant and a period when urediniospores are usually present in abundance in the air. If climatological conditions are favorable—that is, if high relative humidity and comparatively low temperatures prevail during this period—a severe rust epidemic is almost sure to follow if the infection material is present. A study of the climatological conditions during the last of June and the first of July in the spring-wheat belt shows that these conditions existed in the years when rust epidemics were severe and did not exist in the years when rust was not prevalent. These conditions are sufficient to explain any such peculiar behavior as Pritchard refers to and also help to explain rust epidemics in the spring-wheat region.

EXPERIMENTAL DATA

It is seen from the foregoing review that a number of workers have grown rust-infected seed grain under various degrees of isolation and with more or less conflicting results. The evidence of this kind as to the transmission of *Puccinia graminis* by means of seed seems to be largely negative. Nevertheless some positive results have been reported. The one conclusive way to prove the contention that rust can be carried on seed grain must be to produce the disease upon plants grown under controlled conditions from seed known to be infected with the rust. While histological evidence is valuable from the standpoint of interpretation, yet no amount of such work by itself is fully convincing in connecting seed infection with the appearance of the disease upon the leaves unless plants can be grown from infected seed under controlled conditions and the disease be produced upon these plants. The writer's experimental investigations were along three lines: (1) Greenhouse experiments in which rusted kernels of wheat in large numbers were sown under isolated conditions and the resulting plants watched for infection; (2) field experiments in which rusted wheat kernels were sown in the fields and watched to learn if infection occurred upon the resulting plants sooner than upon plants grown from clean seed; and (3) histological investigations in which rusted wheat kernels were germinated under various conditions and the resulting seedlings examined histologically for spread of rust infection from the kernel to the seedling.

GREENHOUSE EXPERIMENTS

The writer determined to test this matter thoroughly by growing a large number of wheat plants from kernels known to bear sori of stemrust, under conditions of isolation and at the same time under conditions normal for the development of the host. In order to meet these requirements a room in the pathological greenhouses at the University of Wisconsin was equipped as shown in Plate 40. The room was examined carefully and every crack and opening sealed. Double doors were constructed with a space between, which could be sprayed each time before the room was entered. An adjustable shade was placed upon the roof in such a way that a spray of water could be thrown upon the glass underneath the shade to aid in cooling the room, and a system of forced circulation of washed air was installed, as shown in figure 1. Thermograph and hygograph records were kept at all times when the plants were growing, and it was found easily possible to control the temperature and humidity within normal limits for growth of wheat plants. Plants grown in this house were entirely normal in appearance and produced plump kernels in every head. The accompanying photographs (Pl. 41) taken at different times during the period when the experiments were in progress, show the normal, healthy condition of the plants. In order to test the efficacy of this air-washing apparatus, about a pint

of smut spores were thrown up into the opening at *i* in figure 1, and an attempt was made to catch any which went through the drum upon moistened sterile cotton held at *h*. This cotton was then washed and the water carefully examined with the microscope. No spores could be found upon this cotton, although the experiment was repeated several times. Every time the room was entered the space between the double

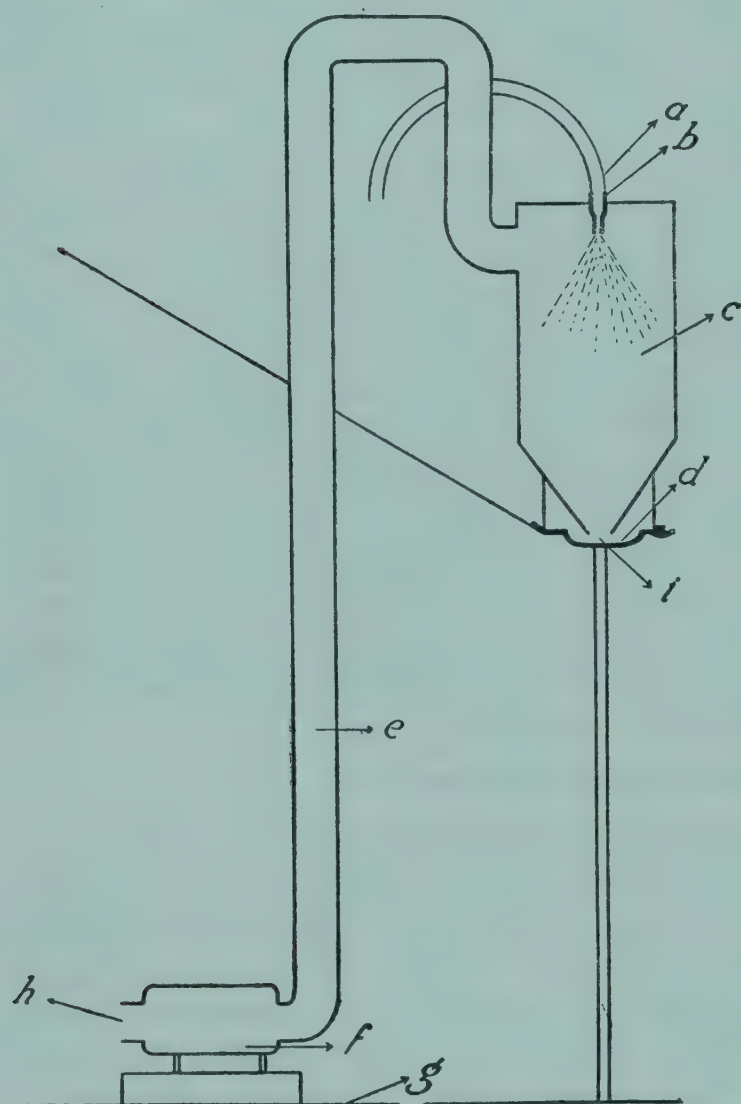


FIG. 1.—Diagram of air-washing apparatus for isolated room used for growing rust-infected seed: (a) Hose connection; (b) spray nozzle; (c) galvanized iron cylinder; (d) greenhouse gutter into which the water from spray drained; (e) connection pipe from cylinder to blower; (f) electric blower; (g) floor of greenhouse; (h) mouth of the blower where air entered the room; (i) air intake.

doors was thoroughly sprayed, and a rubber coat which was kept hanging in this ante-chamber was put on. Although wheat was grown in the adjacent houses and became badly infected with mildew (*Erysiphe graminis*) none appeared on that grown inside of the isolated room. Neither did any aphids, which were plentiful at various times in other rooms in the greenhouse, make their way into the isolated room.

The soil used in these experiments was in every case sterilized, and only boiled water was used for watering until after the lake from which the water supply was derived was frozen over.

Four different lots of rusted seed were grown at different times in this

house. Each lot was sown in flats 12 inches wide, 24 inches long, and 6 inches deep. These experiments will now be considered in the order in which they were performed.

EXPERIMENT I.—Seed for this experiment was selected from lots of wheat obtained from the following sources: Four varieties of durum from the cereal-disease plots at Madison, Wis.; one lot of Marquis from Maynard, Iowa; one mixed lot of wheat from Leith, N. Dak.; one lot of durum from Brookings, S. Dak.; one mixed lot of unknown source from a

grain elevator in Minneapolis, Minn.; one lot of durum from Hagen, N. Dak.; and one mixed lot from Fargo, N. Dak. From all of these wheats rusted seed was selected and sown on November 8, 1915, in the isolated room. Seven hundred and six plants were obtained from this seed and grown to maturity. No rust appeared upon any of the plants at any time. On the primary leaf of two different plants lesions appeared from which cultures of *Helminthosporium* sp. were obtained. No other infection of any kind appeared upon any of these plants. Plate 41, B, shows three flats of plants from this experiment just after the plants were well headed.

EXPERIMENT 2.—Experiment 1 was carried on during the winter months, and it was thought advisable, therefore, to duplicate the work in the spring and sow the seed at the time spring wheat normally would be sown in the field. The same precautions were taken as in experiment 1, and the same room was used. Seed was secured from the following sources: Three lots of mixed seed of unknown source from Minneapolis, Minn.; two lots of mixed seed of unknown origin from Minneapolis, Minn.; three lots of durum from the rust nursery, University Farm, St. Paul, Minn.; one lot of durum from Clark, S. Dak.; two lots of durum from the cereal-disease plots at Madison, Wis.; one lot of Marquis from Maynard, Iowa; one lot of durum from Leith, S. Dak.; one lot of mixed seed from Armour, S. Dak. Rusted kernels from these sources were sown on March 19, 1916, and 730 plants emerged and were grown to maturity. No rust appeared on any of these plants at any time. The experiment was discontinued when the wheat became mature.

EXPERIMENT 3.—The experiment was repeated during the winter of 1916-17, when 760 plants were grown to maturity under the same conditions as outlined above. Seed for this experiment was obtained from various places in North and South Dakota and Minnesota. No rust appeared upon these plants at any time. The experiment was concluded when the plants were mature.

EXPERIMENT 4.—It was thought possible that soil temperatures at the time of the germination of the seed might affect the ability of the fungus to penetrate the young embryo and that the temperature in the isolated room might have been too high for successful infection at the time of germination. In order to simulate more closely natural conditions of germination and growth of the plants, infected wheat kernels were germinated in soil in an Altmann incubator at different temperatures as indicated in Table III.

When the seedlings were about $1\frac{1}{2}$ inches long, they were carefully transferred to pots of sterilized soil and grown in the isolated room until the plants were mature. Twenty-five kernels of wheat were used for each temperature indicated. No rust appeared upon these plants at any time.

TABLE III.—*Temperatures at which infected wheat kernels were germinated*

Date of germination.	Number of plants.	Temperatures.	Date of transfer to greenhouse.
Dec. 12, 1916	21	-2° C. alternated with 15° C.....	Dec. 30, 1916.
Do.....	20	7° C. alternated with 15° C.....	Dec. 26, 1916.
Do.....	20	12° C. continuously.....	Do.
Do.....	23	2° C. alternated with 21° C.....	Do.
Do.....	24	10° C. alternated with 18° C.....	Do.
Do.....	24	15° C. continuously.....	Do.

EXPERIMENT 5.—A number of writers have suggested the possibility of rust infection taking place from spores on the surface of the seed. To test this possibility, several flats of wheat were sown with seed that had been literally covered with viable urediniospores of stemrust. Preston wheat (C. I. No. 3081) was used for this experiment. In all, about 200 plants were grown. No rust infection appeared upon any of them at any time.

FIELD EXPERIMENTS

EXPERIMENT 1.—In the spring of 1916 rusted wheat from various sources was sown in the field along with clean seed and rusted seed treated with the modified hot-water treatment. These plots were examined every few days from the first appearance of rust infection. After June 27 the plants were examined every other day. Table IV gives the methods employed and results obtained in the experiment.

The groups of plots numbered 1 to 4, 5 to 9, 10 to 13, and 14 to 18 were grown in different locations on the University Farm at Madison, Wis.

Stemrust was noted upon *Hordeum jubatum* near two of the plots on July 3, 12 days before it appeared upon the wheat in these plots.¹ Infection also had been common upon adjacent barberries for some time previously. It will be noted that the plants grown from badly rusted samples of seed did not develop rust any earlier or any more severely than those grown from clean seed or from rusted seed which had been treated with the modified hot-water treatment.

Recently the writer has had opportunity to consult the notes on an unpublished experiment somewhat similar to field experiment No. 1, as described above. The work was done by E. C. Johnson, at that time Pathologist in Charge of Cereal Disease Investigations in the Bureau of Plant Industry, and carried on at the University Farm, St. Paul, Minn., in 1912. The experiment is described and results are given in Mr. Johnson's report, a copy of which is on file in the Office of Cereal Investigations, Department of Agriculture, Washington. D. C.

¹ By inoculating wheat plants in the greenhouse this was found to be *Puccinia graminis tritici*.

TABLE IV.—Development of rusts on plants grown in the field from treated and untreated rust-infected seed and rust-free seed in 1916

Plot No.	Description of seed.	Date sown.	Treatment of seed.	Size of plots.	Date on which plants emerged.	Date on which plants headed.	Date of appearance of—		Abundance of infection by—			
							Leaf-rust.	Stem-rust.	Leaf-rust.		Stem-rust.	
									Date.	Per-cent-age.	Date.	Per-cent-age.
1	Marquis, many rusted pieces of glumes, some rust-tipped seed.	May 15	Untreated.	5 rows 10 feet long...	May 20	July 14	June 10	July 14	June 16	1+	July 25	5+
2	do.	do.	Treated.	do.	do.	do.	do.	do.	do.	1+	do.	5+
3	Durum wheat, 1 per cent infection of kernels.	do.	Untreated.	do.	do.	do.	June 12	July 18	do.	1—	do.	Trace.
4	do.	do.	Treated.	do.	do.	do.	June 10	July 15	do.	1	do.	5—
5	Same seed as No. 1.	May 23	Untreated.	5 rows 1 rod long.	May 28	July 20	June 12	do.	June 19	2	do.	3
6	Marquis (C. I. No. 3641), rust-free seed.	do.	do.	do.	do.	do.	do.	July 14	do.	2	do.	5
7	Mixed seed from very badly rusted field, 1 per cent seed infection.	do.	do.	do.	do.	do.	do.	July 15	do.	15	do.	10
8	do.	do.	do.	do.	do.	do.	do.	do.	do.	15	do.	10
9	Preston (C. I. No. 3681), rust-free seed.	do.	do.	do.	do.	do.	June 14	do.	do.	15	do.	10
10	Same as No. 7.	May 3	do.	6 feet by 15 rods.	May 9	July 8	June 6	do.	do.	5	do.	6
11	do.	do.	Treated.	do.	do.	do.	do.	do.	June 16	1	do.	25
12	Durum seed, 1 per cent infection.	do.	Untreated.	do.	do.	do.	do.	July 14	do.	1	do.	20
13	do.	do.	Treated.	do.	do.	do.	do.	July 15	do.	1	do.	20
14	Mixed seed, 1 per cent rusted kernels.	do.	do.	do.	do.	do.	do.	do.	do.	1	do.	20
15	do.	May 18	Untreated.	5 rows 25 feet long.	May 21	July 15	June 12	July 16	do.	5	do.	5
16	Same as No. 3.	do.	Treated.	do.	do.	do.	do.	do.	June 20	7	do.	5
17	Same as No. 1.	do.	do.	do.	do.	do.	do.	do.	do.	3	do.	1—
18	Same as No. 6.	do.	do.	do.	do.	do.	do.	July 15	do.	3	do.	5—
		do.	do.	do.	May 22	do.	do.	July 16	do.	5	do.	8—

a Two sori appeared on one plant in this plot on this date.

Nine different varieties of wheat seed were sown, and the plants were examined for rust every four or five days. Leafrust appeared on all the plots on June 5, and stemrust appeared from July 17 to July 29. Johnson sums up the results as follows: Rusted durum, Fife, and Bluestem kernels produced plants showing no earlier or more severe development of rust than adjacent plants from clean, uninfected seed.

EXPERIMENT 2.—On April 12, 1916, rusted kernels of wheat were sown in separate flats in the greenhouse. About 25 kernels were used from each of the following varieties: Allora (C. I. No. 1698), Kubanka (C. I. No. 1440), and Marquis (C. I. No. 3641). These flats were transferred to the pathological garden May 11, and were at that time in the fifth or sixth leaf. They were headed about June 22, and stemrust did not develop upon them until July 13, when a few leaves of the Marquis wheat, which still remained green, bore sori of *Puccinia graminis*. It will be noted by reference to Table IV that this was about the date upon which stemrust developed upon wheat in the field plots and was indeed about the date when stemrust appeared upon all the wheat in the vicinity. The season was very backward, and rust did not make its appearance nearly so early as usual.

HISTOLOGY OF SEEDS AND SEEDLINGS

HISTOLOGY OF SEED.—The general appearance of the exterior of wheat kernels infected with stemrust has been previously described. In order to examine the interior of these kernels two methods were found to be fairly satisfactory: One, in which the grains of wheat were boiled in water and then sectioned on the freezing microtome; the other, a modification of the glycerin method described by Howard (13). This latter method was found to be satisfactory, and good sections of mature wheat kernels were obtained. After sectioning, Planeze stain was used with good results.

When sections of infected kernels were examined with a microscope it was found that not all the sori appeared upon the surface. In some instances the entire hilar region of the kernel was found to be filled with sori, of which from 1 to 12 were found in a single kernel. These sori often were found facing inward against the aleurone layer which was very much distorted by the pressure (Pl. 42). Other sori were found, nearly spherical in form, entirely embedded in the pericarp tissue. There seemed to be no regular arrangement, although the sori were often arranged in a circle around the hilum. This is what would be expected, for many of them undoubtedly were connected with infection on the rachilla before the kernel was broken away from the point of attachment. Plate 43 is a longitudinal section through the hilum of an infected kernel and shows the hilum nearly cut off by a large sorus, which probably was formed from several sori that had become confluent. Plate 44 is a cross

section of a mature wheat kernel with telia upon the ventral surface. Plate 45 is an enlarged portion of the same.

Internal rust sori of wheat kernels were noted and described also by Pritchard (22). More recently Colley (8) has listed 11 reports of internal rust sori upon various hosts. He concludes that these are rather common teratological phenomena having no especial morphological significance and can be expected to occur whenever the point at which the sorus begins to form is located beneath a layer of tissue which is too resistant for the sorus to break through. Plates 46 and 47 also show internal sori.

HISTOLOGY OF SEEDLINGS.—Rusted kernels of wheat were germinated under various conditions and for various lengths of time. These were fixed, sectioned, and examined for spread of infection from mycelium or spores embedded in the tissues. Various materials were used for fixing these young seedlings, but it was found that Juel's fixative penetrated the embryonic parts better than any other which was tried, although Fleming's medium fixative gave fairly satisfactory results. After sectioning, either triple stain with excess of Orange G or Pianeze stain was found to be satisfactory for differentiating host and fungus tissue.

Infected seed was germinated under the following conditions. Seed from lot 1 was germinated in compartments of an Altmann incubator kept at 2°, 12°, and 17° C., respectively. Part of these were fixed when the plumule was about $\frac{1}{2}$ inch long, and the rest when the first leaf was just beginning to unfold. Seed from lot 2 was germinated in compartments of the Altmann incubator at temperatures of 2° alternated with 17° and 11° alternated with 21°. The experiments with lots 1 and 2 were conducted twice—once in November, 1915, and again in April, 1916, after the infected seed had been kept in a cool place over winter. Lot 3 was sown in pots which were placed in small chambers in the greenhouse where the soil temperature was kept between 11° and 15° by the use of ice. When the plants were about 3 or 4 inches tall they were fixed, and a portion of each was sectioned and examined. Lot 4 was germinated and buried out of doors in the ground at seeding time in the spring. The plants were treated as were those in lot 3.

Hundreds of sections were prepared from the material described above. In no case was there any positive evidence of spread of infection from the infected seed to the young plant.

Plates 46 and 47 illustrate this fact. Plate 46 represents a longitudinal section through a wheat embryo in a very early stage of development. There is no indication of any spread of rust mycelium from the sori seen in the infected hilar region at *x*. Plate 47 also represents a longitudinal section of a wheat embryo. In this case development has progressed considerably further than that shown on Plate 46. There is, however, absolutely not the slightest indication of spread of rust mycelium from the large sorus shown at *x*.

From all appearances the rust mycelium was dead in the sori of the germinated kernels shown in Plates 46 and 47. The same was true of the rust mycelium in wheat kernels that had been stored for some time. All such mycelium was devoid of normal protoplasmic content. This fact together with the apparent inability of this mycelium to spread to the developing seedling indicates clearly to the writer that this mycelium was dead. In fact, only in fresh kernels which were not fully matured were any living rust mycelia found. Numerous efforts were made also to germinate the teliospores found in sori upon the hilar portions of wheat kernels, but all were unsuccessful.

Hyphae of other organisms were present in abundance everywhere in the pericarp of many kernels and in some cases were found to penetrate the embryo. These hyphae were much larger and of an appearance different from the rust hyphae found at the base of the sori in the hilar portions of the kernels, as previously described. They penetrated directly through the cell walls of the host and broke down the cell structure to a much greater extent than rust infection was found to do. Plate 48 shows an oblique longitudinal section of a secondary root of a wheat seedling being invaded by this type of parasite. This was probably some species of *Helminthosporium*, for typical *Helminthosporium* spores were found on the germ end of the kernel from which this section was made. Mycelium of the same type was found in the root, stem, and sheath of a number of seedlings which were grown from kernels of wheat having a distinct browning of the hilar ends somewhat similar to the general appearance of rust-infected kernels. It seems not entirely unlikely, therefore, that the apparently similar mycelium referred to by Pritchard (21) may have been of this type, especially since he states that the mycelium he noted also was intracellular.

The writer did not find any "palmella-like" developments from the teliospores, as described by Pritchard. However, no seed over 1 year old was used, and since Pritchard used seed 5 years old this may to some extent account for the difference.

SUMMARY

(1) Uredinia and telia of *Puccinia graminis tritici* Erikss. and Henn. have been found embedded in the pericarp on the hilar end of kernels of wheat and sometimes along the ventral groove as far up as the middle of the kernel. Infected kernels have black hilar ends, and groups of telia appear as shining black specks under either the hand lens or the binocular microscope.

(2) Only a small percentage of infection was found by examination of the hundreds of samples of wheat from the crops of 1915 and 1916. A little over 1 per cent was the largest quantity found in any sample. The durum wheats were found most commonly infected.

(3) Infection undoubtedly spreads to the kernel from original infection on the rachis, rachilla, or glumes.

(4) The germinating power of the seed apparently is not impaired by this rust infection.

(5) When rusted kernels of wheat were sown in the field, no earlier or more severe rust infection occurred on the resulting plants than on those grown in adjacent plots which were sown either with clean seed or with rust-infected seed which had been treated with the modified hot-water treatment.

(6) More than 2,500 plants were grown from rusted seed in a specially constructed room in the pathological greenhouse at the University of Wisconsin, and no rust infection appeared upon any of them at any time. The conditions of growth of these plants were normal, and they produced plump grain.

(7) No spread of infection from the pericarp to the young plant was found by examination histologically, although infected seed were germinated under various conditions, simulating as nearly as possible natural conditions in the field.

(8) No infection appeared upon plants grown from seed which had been covered with viable urediniospores of stemrust before sowing.

(9) The results of the experimental work here reported indicate that stemrust is not transmitted from one wheat crop to the next by means of infected seed grain. Further, in the writer's judgment, the occurrence of stemrust sori in the pericarp of the caryopses of grains and grasses has no especial significance, but the infection spreads to these tissues just as it does from an infection point in any of the vegetative parts of the plant.

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PLATE 38

Heads of Kubanka durum wheat heavily infected with stemrust. Collected at Dickinson, N. Dak., in 1916.

(278)



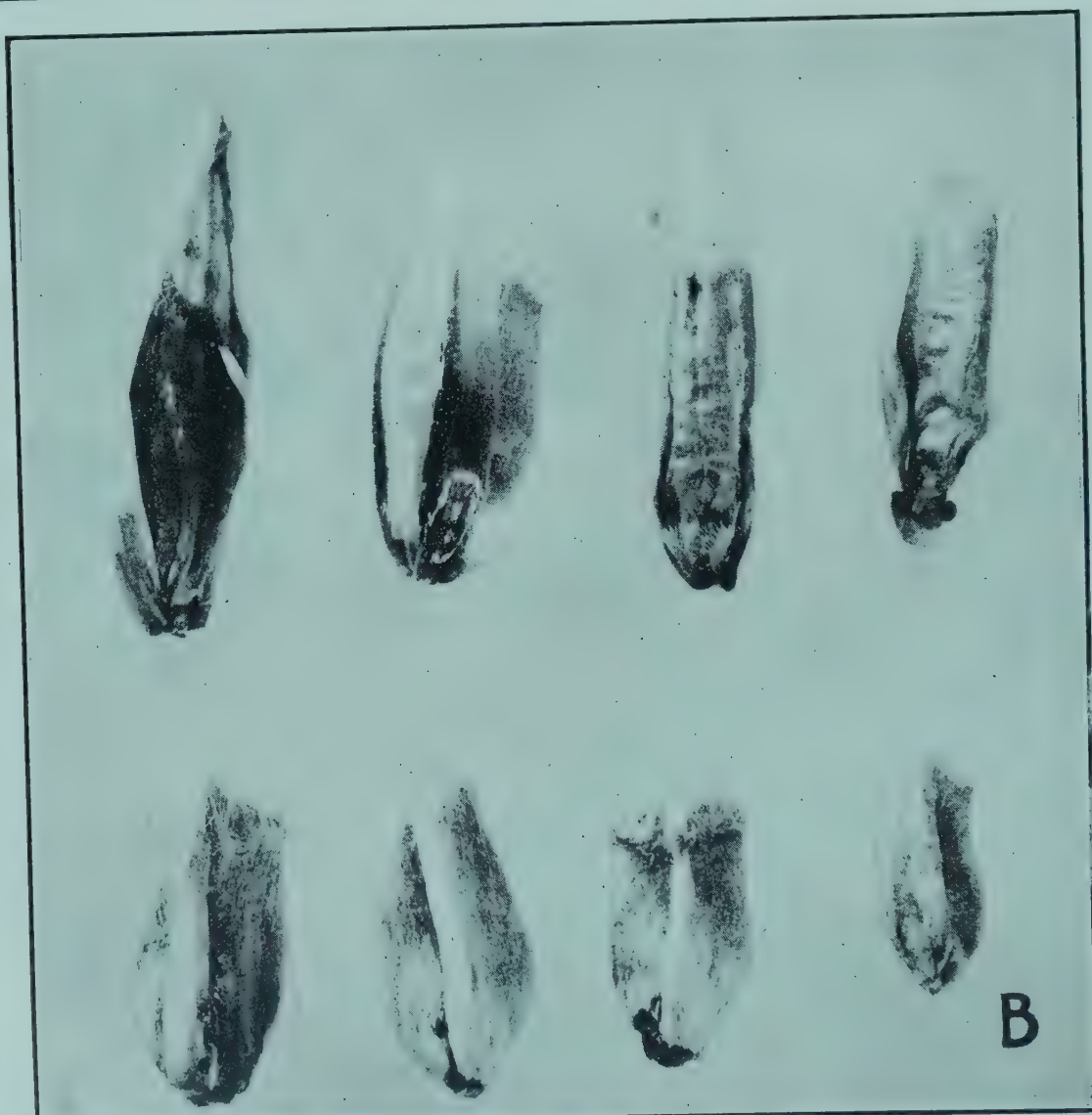
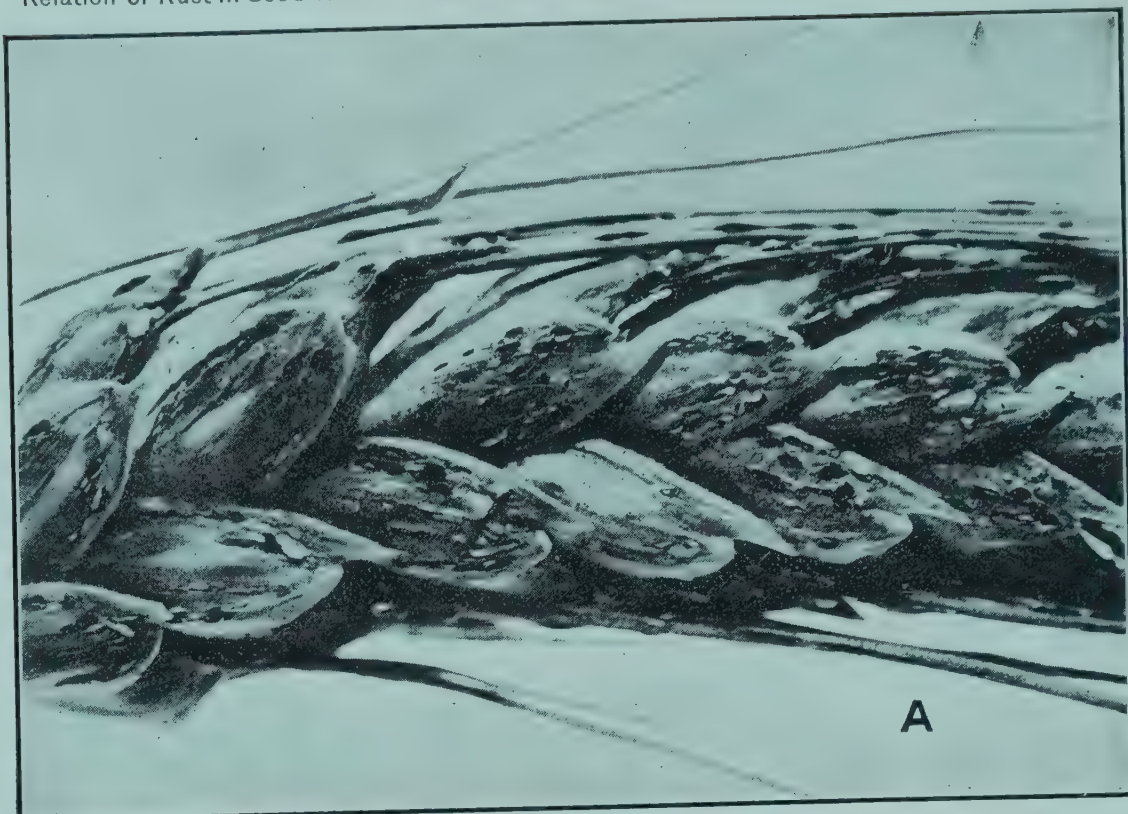


PLATE 39

A.—Portion of one of the heads shown in Plate 38. $\times 3.6$.

B.—Wheat kernels showing typical stemrust infection. Abundant infection occurs at the base of the attached paleae on the upper row of kernels. In the lower row rust sori occur at the hilar end and along the ventral groove. $\times 6$.

PLATE 40

Exterior view of isolated room in the pathological greenhouse at the University of Wisconsin, showing (*a*) the exterior portion of air-washing apparatus used to wash all air drawn into the room, (*b*) the canvas curtain used for shading on warm days, and (*c*) the sprinkling attachment used to throw spray of water over the roof to aid in keeping the room cool. (See fig. 1 and description of apparatus in text.)

A.—Greenhouse with canvas curtain rolled up.

B.—Greenhouse with canvas curtain rolled down.

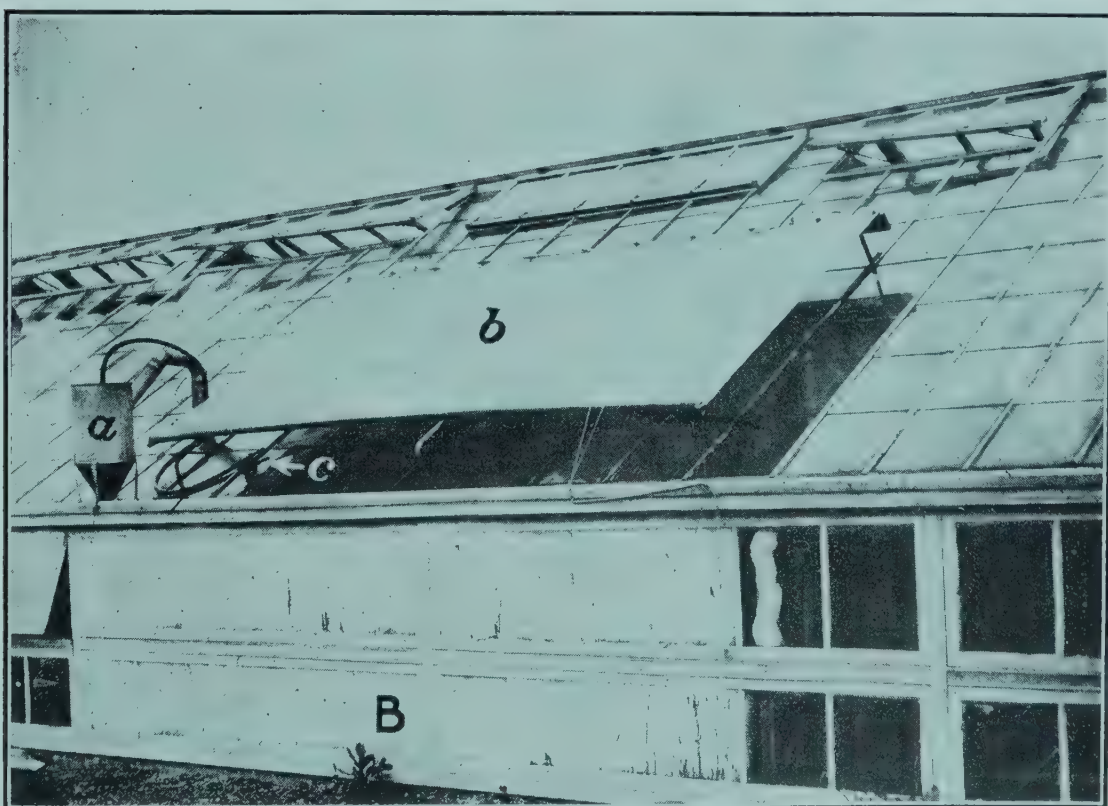




PLATE 41

A.—Photograph of wheat grown in flats in isolated room in greenhouse at the University of Wisconsin. The healthy, vigorous growth of the plants indicates that normal growing conditions prevailed in the greenhouse.

B.—Same plants as A, when well headed. Plump kernels of wheat were harvested from all these plants.

PLATE 42

Longitudinal section through hilar portion of an immature wheat kernel, showing sorus of stemrust. Abundance of living rust mycelium is shown at the base of the sorus. Note (at left) the aleurone layer which has been forced inward. No evidence of mycelial penetration into aleurone layer of cells. $\times 245$.

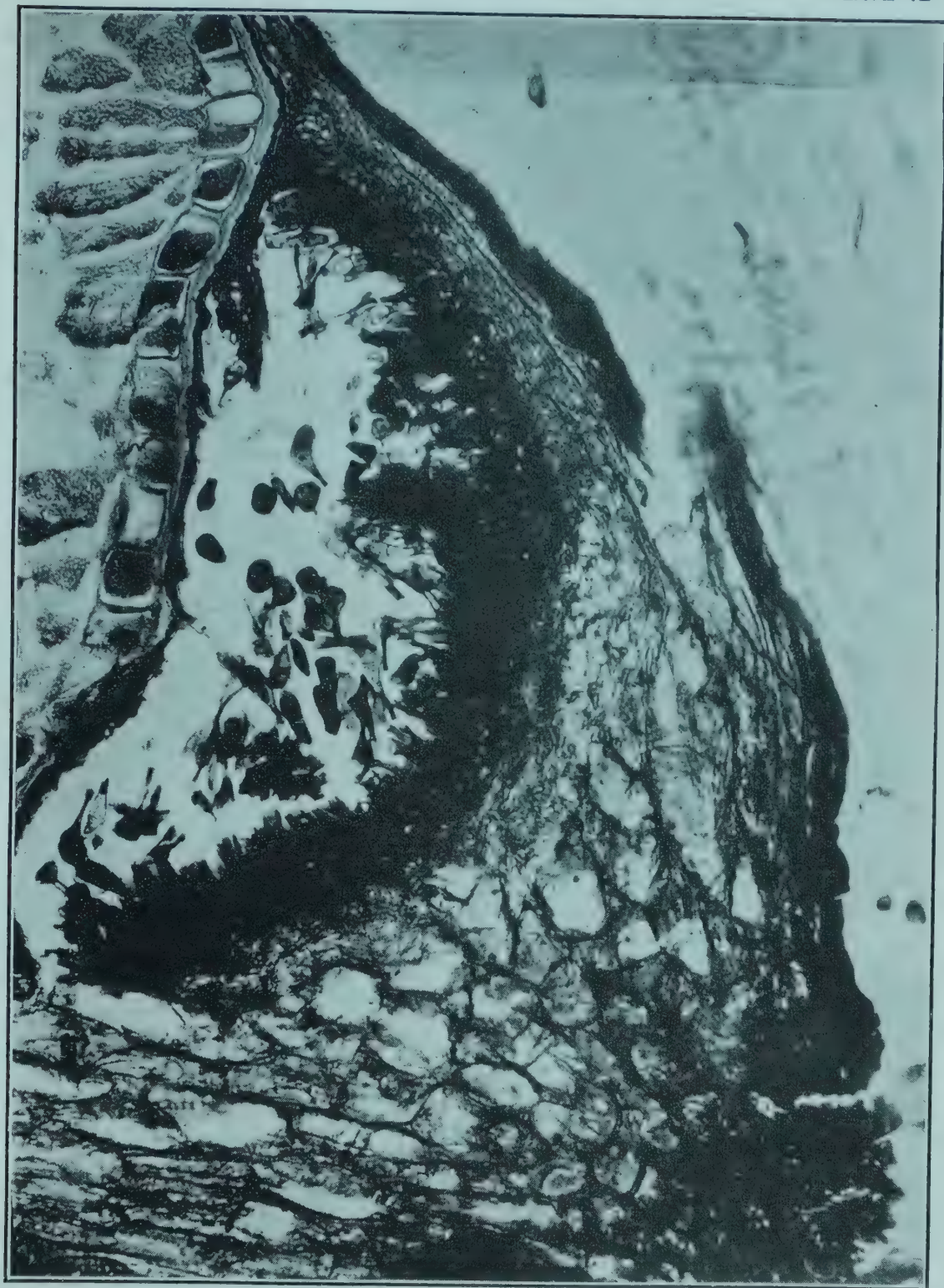




PLATE 43

Longitudinal section through the hilum of a wheat kernel infected with stemrust, showing unusually large internal sori extending nearly across the kernel. Both external and internal sori are shown. No evidence of invasion of aleurone cells was found. $\times 85$.

PLATE 44

Cross section of a mature wheat kernel infected with stemrust, showing telia in the ventral groove. Note the normal appearance of the cells of the aleurone layer immediately beneath the sori. $\times 50$.





PLATE 45

Enlarged portion of section shown in Plate 44, showing telia on surface of ventral groove. No evidence of penetration into aleurone cells exists. $\times 278$.

PLATE 46

Longitudinal section of embryo of germinated wheat kernel showing large internal rust at x in hilar tissue at base of embryonic tissue. Hundreds of such sections were examined without evidence of spread of rust infection to the embryo. $\times 65$.

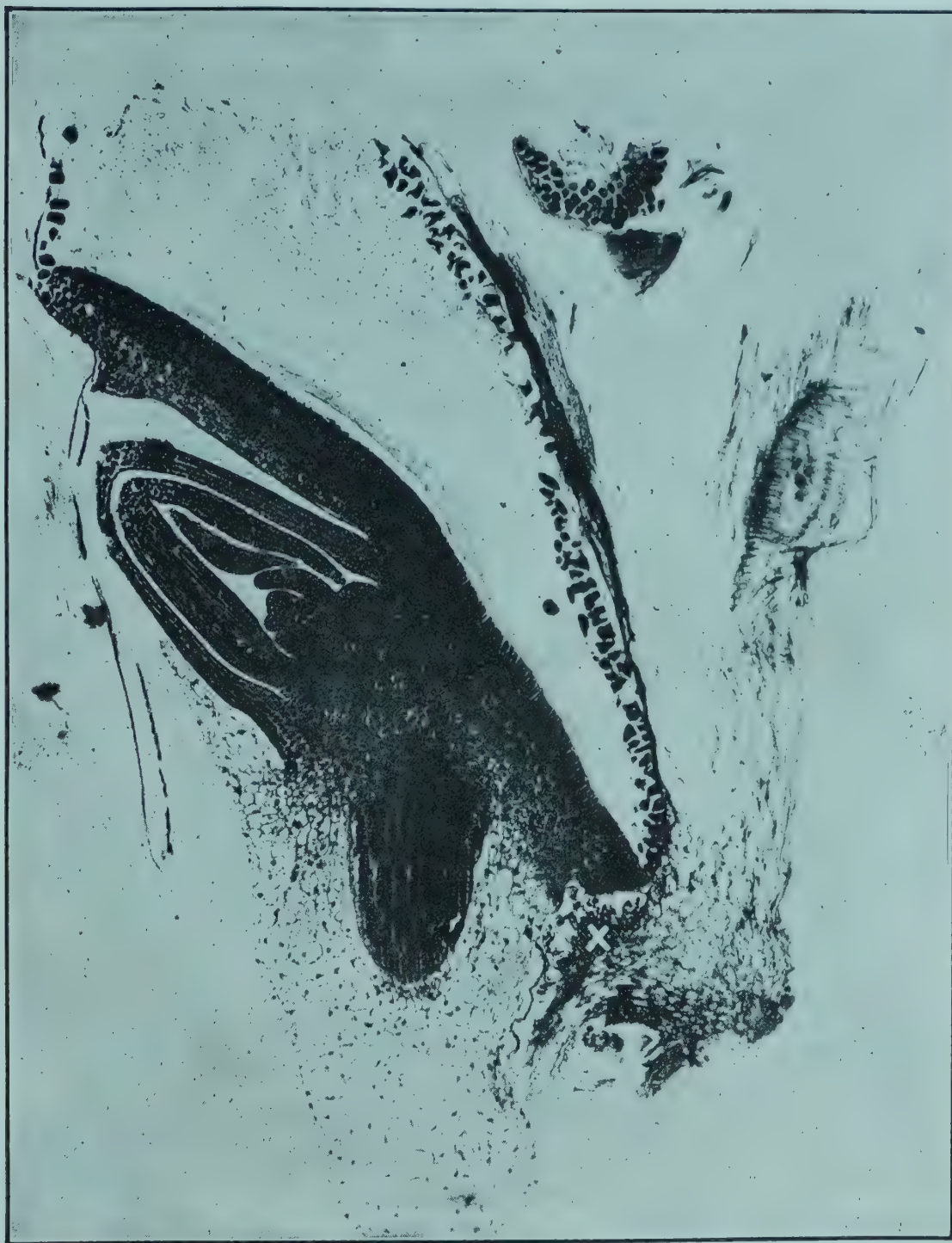




PLATE 47

Longitudinal section of the embryo further advanced in development than that shown in Plate 50. Internal hilar sorus shown at *x*. No evidence of infection of embryonic tissues. $\times 67$.

175344°—20——4

PLATE 48

Longitudinal section through young secondary root of wheat embryo, showing presence of intracellular mycelium. The fungus here is probably a species of *Helminthosporium*. This mycelium is larger and more vacuolated and breaks down the cells of the host much more completely than does the rust mycelium. (See Pl. 42 for comparison.) $\times 255$.



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PRACTICAL UNIVERSALITY OF FIELD HETEROGENEITY AS A FACTOR INFLUENCING PLOT YIELDS

By J. ARTHUR HARRIS

*Collaborator, Office of Western Irrigation Agriculture, Bureau of Plant Industry,
United States Department of Agriculture*

INTRODUCTION

With the development of a more intensive agriculture there must be a wider use and a progressive refinement of the method of plot tests in agronomic experimentation. Betterment of the method of plot tests must be sought along two lines, (1) the perfection of biological technic and (2) the more extensive use of the modern higher statistical methods in the analysis of the results.

In 1918 Mr. C. S. Scofield, in charge of the Office of Western Irrigation Agriculture, and Prof. E. C. Chilcott, in charge of the Office of Dry-Land Agriculture, asked the writer to undertake an investigation of the statistical phases of the problem of the accuracy of plot tests. The present paper deals with one aspect only of the general problem, that of the lack of uniformity of the experimental field. This is both the most potent cause of variation in plot yields and the chief difficulty in their interpretation.

Many of the careful writers on field experimentation have noted the existence of soil heterogeneity. Few have, however, sufficiently recognized and none have adequately emphasized the importance of this factor.

The problem of field heterogeneity is twofold. First, some measure of the amount of its influence upon crop yields must be obtained. Second, some means of avoiding or of correcting for its influence must, if possible, be secured.

An exact measure of the influence of field heterogeneity, and not merely a vague notion that it may influence experimental results, is the first and most fundamental step in the closer analysis of the factors determining the variability of plot yields. If the application of such a criterion to results obtained by practised agriculturalists from fields selected for their uniformity shows no evidence of heterogeneity, plot tests may be carried out along conventional lines with confidence that

with reasonable precautions reliable results will be obtained. If, on the other hand, the application of such a criterion shows a high degree of irregularity in fields selected for their uniformity by experienced agriculturalists, it is evident that very special precautions must be taken to obtain trustworthy results. Some quantitative measure, and some probable error of this measure, of the amount of irregularity of the soil of a field, as shown by actual capacity for crop production, and not merely a demonstration of its existence is, therefore, required.

The purpose of this paper is to show by the analysis of the actual yields of test plots reported by agricultural experts that the securing of fields suitable for a direct comparison of yields is, practically speaking, an impossibility. The results show that unless special precautions are taken irregularities in the field may have greater influence upon the numerical results of an experiment than the factors in crop production which the investigator is seeking to compare.

The results of this study may seem to be altogether negative—destructive rather than constructive. The unbiased student must, however, admit that a full evaluation of all the sources of error is an essential prerequisite to constructive work. Furthermore, large expenditures of public funds are being devoted to fertilizer tests, variety tests, and rotation experiments. It is preeminently worth while to ascertain to what extent results derived from methods now in use may be considered reliable.

Subsequent papers will treat other phases of the problem.

FORMULAE

A criterion of field homogeneity (or heterogeneity) to be of the greatest value should be universally applicable, be comparable from species to species, character to character, or experiment to experiment, and be easy to calculate.

In 1915 the suggestion was made (5)¹ that we may proceed as follows: Suppose a field divided into N small plots, all sown to the same variety of plants. Let p be the yield of an individual plot. The variability of p may be due purely and simply to chance, since the individuals of any variety are variable and the size of the plots is small, or it may be due in part to the diversity of conditions of the soil. If irregularities in the experimental field are so large as to influence the yield of areas larger than single plots,² they will tend to bring about a similarity of adjoining plots, some groups tending to yield higher than the average, others lower.

Now let the yields of these units be grouped into m larger plots, C_n , each of n contiguous ultimate units, p . The correlation between the

¹ Reference is made by number (*italic*) to "Literature cited," p. 313-314.

² Irregularities of soil influencing the plants of only a single small plot may in most work be left out of account, since they are of the kind to which differences between individuals are to a considerable extent due and are common to all the plots of a field.

p 's of the same combination plot, C_n , will furnish a measure (on the scale of 0 to ± 1) of the heterogeneity of the field as expressed in capacity for crop production. If this correlation be sensibly 0 (under conditions such that spurious correlation is not introduced), the irregularities of the field are not so great as to influence in the same direction the yields of neighboring small plots. As heterogeneity becomes greater the correlation will also increase. The value of the coefficient obtained will depend somewhat upon the nature of the characters measured, somewhat upon the species grown, somewhat upon the size of the ultimate and combination plots, and to some degree upon the form of the combination plots.

Knowledge of the values of the correlations to be expected must be obtained empirically.

Let S indicate summation for all the ultimate or combination plots of the field under consideration, as may be indicated by C_n or p . Let \bar{p} be the average yield of the ultimate plots and σ_p their variability, and let n be constant throughout the m combination plots. Using the formulae of an earlier memoir (3) in a notation which is as much simplified as possible for the special purposes of this discussion,

$$r_{p_1 p_2} = \frac{\{[S(C_n^2) - S(p^2)] / m[n(n-1)]\} - \bar{p}^2}{\sigma_p^2}.$$

This formula assumes the combination plots to be of uniform size—that is, to contain each the same number, n , of ultimate plots. It may be desirable or necessary to have some of the combination plots smaller than the others.

Such cases are frequently met in practical work. For example, the wheat field of Mercer and Hall is laid out in a 20 by 25 fold manner. This permits only 2 by 5, 4 by 5, or 5 by 5 combinations of the same size throughout. One of Montgomery's experiments with wheat covered an area of 16 by 14 plots which may be combined in only 2 by 2 or 4 by 2 fold groupings to obtain equal areas suitable for calculation. In each of these cases other groupings are desirable.

The formulae are quite applicable to such cases; the arithmetical routine is merely a little longer. The formula is as above, but \bar{p} and σ_p are obtained by a $(n-1)$ -fold weighting of the plots,¹ where n is the variable number of ultimate plots in the combination plot to which any p may be assigned—that is,

$$\bar{p} = S[(n-1)p] / S[n(n-1)],$$

$$\sigma_p^2 = \frac{S[(n-1)p^2]}{S[n(n-1)]} - \left(\frac{S[(n-1)p]}{S[n(n-1)]} \right)^2.$$

¹ That is, each ultimate plot is multiplied by the number less one of the plots in the combination plot to which it is assigned.

Ample illustration of the arithmetical routine has been given in the original paper.

The formulae employed assume the symmetry of the correlation surface. It has been shown elsewhere (4) that spurious values of the correlation coefficient may arise in such cases. Since both $\bar{p}_1\bar{p}_2$ and $\sigma_{p_1}\sigma_{p_2}$ take the maximum values when, because of the symmetry of the correlation surfaces, $\bar{p}_1=\bar{p}_2$, $\sigma_1=\sigma_2$, it is clear that the limiting value of the spurious correlation will be 0.

Thus it is possible that heterogeneity exists even when $r_{p_1p_2}=0$, but a field can not be considered homogeneous if $r_{p_1p_2}$ has a value which is statistically significant in comparison with its probable error.

Practically, little difficulty will arise from this source, and it can usually be easily avoided by the exercise of a little care in the selection of the proper grouping in doubtful cases.

According to the foregoing conception the relationship between the yield of associated plots is expressed on the universally comparable scale of r , ranging from 0 to ± 1 .

When symmetrical tables are used—that is, when each plot is used once as a first and once as a second member of the associated pair— $\bar{p}_1=\bar{p}_2$, $\sigma_{p_1}=\sigma_{p_2}$, and the regression slope is identical with the correlation coefficient.

Thus, if one ultimate plot, p_1 , of a combination plot be known, the most probable deviation of another plot will be $p_2-\bar{p}=(p_1-\bar{p})r$.

Concretely, if the yield of a first plot of a combination plot be 10 pounds above the average of the field as a whole and if the interplot correlation be $r_{p_1p_2}=0.60$, the most probable yield of a second plot will be 6 pounds above the average.

Similar reasoning applies throughout. Those who have difficulty in thinking in terms of correlation coefficients can most easily grasp the significance of the results by remembering that in this case the correlation coefficients multiplied by 100 gives the most probable percentage of deviation of the yield of an associated plot when the deviation of one plot of the group from the general average is known.

INFLUENCE OF SOIL HETEROGENEITY ON YIELD OF FIELD CROPS

In the paper in which these formulae were suggested it was shown that yield of straw and grain and the nitrogen content of wheat, yield of roots and tops of mangolds, and yield of timothy hay are markedly influenced by irregularities in the carefully selected fields upon which plot cultures have been carried out by agriculturalists.

We have now to ascertain whether this is a general phenomenon or whether it is merely a chance result of these particular cultures. The suggestion has been made that the latter is the case, that with the exercise of a little care uniform fields may be secured, and that substratum

heterogeneity was overemphasized as a factor influencing plot tests. This question can be answered only by actually determining the degree of heterogeneity existing in the fields which have passed the criticism of agricultural experts.

It will be conducive to brevity to have a definite system by which the arrangement of the plots in a field may be described. We shall consider the plots arranged as soldiers in ranks and files. The worker inspects the plot records of a field as recorded on a map or table. By ranks we understand the horizontal rows of plots, by files the vertical rows.

1.80	1.83	2.00	1.91	1.90	1.89	1.79	1.75	2.03	1.83	2.18	1.93	1.77	1.86
1.80	2.07	1.77	1.90	1.70	1.79	1.90	2.04	1.95	1.83	2.06	1.76	1.86	1.79
1.93	1.96	1.83	1.92	1.69	1.90	1.80	1.89	1.83	1.85	2.00	2.13	1.82	1.83
1.89	1.96	1.92	1.86	1.79	1.86	1.79	1.94	1.92	1.80	1.97	2.00	1.87	1.73
2.00	2.01	1.89	1.77	1.97	1.85	1.97	2.10	1.99	1.83	2.00	1.92	1.79	1.89
1.96	1.96	2.00	1.82	1.93	1.82	1.87	1.87	1.92	1.99	1.87	1.83	1.92	1.96
1.89	2.11	1.99	1.87	1.86	1.84	2.06	1.90	1.90	1.82	1.81	1.97	1.79	1.89
2.03	1.86	1.80	1.86	2.06	1.72	1.86	1.72	2.07	1.82	1.84	1.97	1.96	2.01
1.83	1.82	1.82	1.75	1.77	1.72	1.90	1.83	1.90	1.83	1.90	1.85	1.76	2.07
1.87	2.14	1.96	1.87	1.97	1.90	1.90	2.13	1.80	1.83	1.90	2.06	1.94	1.87
1.90	1.94	1.94	1.77	1.89	1.86	1.82	1.87	1.80	1.84	1.87	2.04	1.94	1.89
1.94	1.76	1.96	1.99	1.87	2.04	1.93	1.77	1.74	1.89	1.93	1.96	2.04	1.97
1.83	1.99	1.97	2.08	1.99	1.96	2.15	1.82	1.78	1.83	1.98	1.89	1.85	1.87
1.85	1.87	1.85	1.82	1.92	1.89	2.13	1.82	1.73	1.83	1.96	2.04	1.86	2.08
2.10	1.83	1.85	1.96	2.01	1.92	1.68	1.89	1.85	1.85	1.83	1.85	2.07	1.75
1.93	1.86	1.93	1.87	1.90	1.86	1.99	1.89	1.83	1.82	1.96	1.99	1.99	2.06

FIG. 1.—Montgomery's diagram of 5.5 by 5.5 foot plots of Turkey wheat, showing variations in the percentage of nitrogen in the grain.

Thus figure 1, showing the nitrogen content of wheat plots 5.5 by 5.5 feet given by Montgomery (17), may be considered made up of 16 ranks and 14 files.

In considering rearrangements or combinations of plots we shall refer to the ranks and then to the files—an order easily carried in mind by remembering the trite expression "rank and file." Thus in referring to a 2 by 5 fold combination we mean that two adjacent ranks and five adjacent files of plots were combined. Individual plots may be easily designated. Thus, the plot belonging to the sixth rank¹ and the fifth file in the nitrogen contents of wheat yields contained 1.93 per cent nitrogen.

¹ Ranks are numbered from the top of map, files from the left.

I.—MANGOLDS

The yields of 200 plots of mangolds studied by Mercer and Hall (15) may be grouped into combination plots in a 2 by 2 fold manner. When this is done, the correlation between the yields of associated plots has been shown¹ to be as follows:

For weight of roots, $r = 0.346 \pm 0.042$,² $r/E_r = 8.24$.

For weight of leaves, $r = .466 \pm .037$, $r/E_r = 12.5$.

Thus, if one plot of a combination plot is higher or lower than the general average by a given amount, an associated plot may be expected to deviate from the general average by 35 to 40 per cent of this amount.

2.—POTATOES

Lyon (14) gives the yield in pounds for each of six sections of a series of 34 rows of potatoes. This crop was harvested from "a piece of apparently uniform land." Each section was 72 feet 7 inches in length. The distance between rows was 34 inches.

Combining yields of rows and of sections of rows by twos, we reduce the field from a 34 by 6 fold to a 17 by 3 fold combination. The correlations between the sections of the rows is then found to be

$$r_{p_1 p_2} = 0.311 \pm 0.043, r/E_r = 7.30.$$

Yield of potatoes in this field is, therefore, markedly influenced by irregularities of soil conditions.

For data on a second test on the influence of field heterogeneity on the yield of potatoes we may avail ourselves of the valuable records of yields of individual hills reported by Stewart (19). Since these are recorded in quadruplets for the purpose of determining the influence of missing hills upon yield,³ it is not feasible to group them into plots. The influence of heterogeneity may be tested by determining the correlation between the yields of the plants of a quadruplet.⁴

¹ For original data see Mercer and Hall (15, p. 109); also Harris (5, p. 434-436).

² The probable errors have in all cases been computed on the basis of the actual, not of the weighted, number of ultimate plots as N .

³ The planting scheme adopted was

$$0 \quad a_1 \quad a'_1 \quad b'_1 \quad b_1 \quad 0 \quad a_2 \quad a'_2 \quad b'_2 \quad b_2 \quad 0 \quad a_3 \quad a'_3 \quad b'_3 \quad b_3 \quad . \quad . \quad .$$

where a and a' are the two halves of the same tuber and b and b' are two halves of another tuber. Thus halves a and b were grown adjoining missing hills and were subject to competition on one side only, whereas halves a' and b' were subject to competition from two adjacent plants.

⁴ Since a and a' are halves of the same tuber and b and b' are halves of another, the correlations raa' , rbb' might be due to a specific physiological influence of the characters of the tuber upon both plants developing from the corresponding half tubers rather than to an influence of differences in soil conditions. We have, therefore, determined the correlations between the plants occupying the same relative position in the quadruplet but derived from different parent tubers, that is rab , $ra'b'$. Hence rab represents the correlation between the two outside tubers and $ra'b'$ the correlation between the two inside tubers of the quadruplet. As a control on the results the correlations between one outside and one inside plant have been determined. These are rab' and rba' .

The data given by Stewart are number of tubers and total weight of tubers per plant. These two characters permit the determinations of the average weight per tuber.

When all the pairs are omitted which have been omitted by Stewart¹ or have been designated as affected by leafroll, there remain 139 quadruplets. Determining the correlations between the yield of the two plants derived from different tubers but exposed to the same conditions for growth, we have the following correlations:

For number of tubers per hill—

$$r_{ab} = 0.318 \pm 0.051, r/E_r = 6.19.$$

$$r_{ab'} = .138 \pm .056, r/E_r = 2.46.$$

$$r_{a'b} = .230 \pm .054, r/E_r = 4.26.$$

$$r_{a'b'} = .220 \pm .054, r/E_r = 4.04.$$

For total weight of tubers per hill—

$$r_{ab} = 0.457 \pm 0.045, r/E_r = 10.10.$$

$$r_{ab'} = .312 \pm .052, r/E_r = 6.00.$$

$$r_{a'b} = .427 \pm .047, r/E_r = 9.09.$$

$$r_{a'b'} = .290 \pm .052, r/E_r = 5.53.$$

For average weight of tubers—

$$r_{ab} = 0.237 \pm 0.054, r/E_r = 4.39.$$

$$r_{ab'} = .104 \pm .057, r/E_r = 1.82.$$

$$r_{a'b} = .054 \pm .057, r/E_r = .95.$$

$$r_{a'b'} = .117 \pm .056, r/E_r = 2.07.$$

The correlations are positive throughout and generally statistically significant with regard to their probable errors. They show, therefore, that this experimental plot was heterogeneous to an extent that influenced in a very measurable degree the number of tubers, the total weight of tubers, and the average weight of tubers of neighboring hills. For all four measures of interdependence the coefficients are lowest for average weight of tubers and highest for total weight of tubers, while the correlations for number of tubers produced are intermediate in value.

The values of r_{ab} are consistently higher than those for $r_{a'b'}$, notwithstanding the fact that a' and b' are more closely associated than a and b . The measures of interrelationship between the yields of pairs of plants, one of which occupies an inside and the other an outside position in the quadruplet, are sometimes intermediate between r_{ab} and $r_{a'b'}$ and sometimes less than $r_{a'b'}$. On the assumption that the correlation is due solely to environmental influence one would expect the highest

¹ Records have been abstracted from Stewart's Table I. Prof. Stewart has kindly furnished some additional information in regard to certain entries in this table.

correlation between the most closely associated plants—that is $r_{a'b'} > r_{ab}$. Apparently the reverse condition, $r_{a'b'} < r_{ab}$, is due to some

influence of the open space adjoining *a* and *b*, which allows the fuller development of those plants and in consequence renders them more representative of the extremely localized soil influences to which they are subjected.¹

3.—TIMOTHY HAY

The records of plot yields of timothy hay published by Holtsmark and Larsen (8) have been shown elsewhere (5) to present a correlation between the yield of ultimate plots, combined in a 2 by 2 fold manner, of

$$r = 0.611 + 0.027, r/E_r = 22.4.$$

Clearly the field was highly heterogeneous.

4.—ALFALFA HAY

Records of the yields of a series of 46 plots on the Huntley Experiment Farm, Montana, may be used to test further the influence of heterogeneity on the yields of alfalfa hay. Data were kindly placed at my disposal by Mr. C. S. Scofield.

Alfalfa should be of especial interest in the present discussion since it is a deep-rooted perennial herb, whereas all other herbaceous crops investigated have been annuals, or at most biennials.

In field B of this experimental farm there are two series, II and III, each of 23 plots. The 46 plots form a solid block which has been planted each year to one crop just as if it were an ordinary field.

The two series of plots are separated from each other only by a temporary irrigation ditch. Each plot is $23\frac{1}{3}$ feet wide, 317 feet long, and contains approximately 0.17 acre. These plots have in certain

III		II	
b	a	b	a
230	305	290	305
180	290	240	290
200	310	300	340
210	265	285	355
200	260	300	325
225	285	280	345
215	285	275	365
220	235	270	285
255	235	285	285
210	230	280	260
240	245	300	285
235	235	265	265
230	270	270	295
210	260	270	285
225	260	315	340
225	235	320	330
220	240	275	315
230	200	285	350
255	225	295	340
265	255	310	295
235	225	320	305
250	280	310	315
240	265	310	280

FIG. 2.—Diagram showing yield of alfalfa in first cutting, 1913, on the Huntley experimental tract. The yield is expressed in pounds per half plot.

cases been harvested in subplots of 0.085 acre when the division has been into halves, of 0.0567 acre when the division has been

¹ Possibly competition between closely associated *a'* and *b'* plants tends to make the yield of one low when that of the other is high.

into thirds, and of 0.0425 acre when the division has been into quarters of plots.

In the spring of 1912 the whole field was uniformly seeded to alfalfa; only one crop was harvested, and yields were recorded for the entire

III				II			
b		a		b		a	
70	95	125	135	135	155	135	175
110	75	85	160	145	125	125	165
80	90	125	110	165	155	150	160
100	65	130	130	145	180	145	180
115	95	110	125	135	165	100	140
115	125	135	135	125	185	130	155
110	95	120	115	145	175	100	155
120	90	100	115	140	150	100	180
100	90	80	105	125	150	45	150
95	95	105	120	125	140	60	145
115	80	95	100	120	140	65	110
115	90	90	105	125	145	120	60
110	100	110	130	120	140	110	115
115	85	120	165	130	150	100	130
105	105	100	145	130	150	145	140
150	95	100	95	100	150	110	115
135	115	90	105	95	110	100	130
155	125	120	100	65	130	115	115
145	130	145	95	120	120	100	115
170	135	155	105	95	135	95	115
135	125	155	95	110	120	115	110
140	115	160	120	110	145	115	130
150	100	120	160	85	150	105	85

FIG. 3.—Diagram showing yield of alfalfa in second cutting, 1913, on the Huntley experimental tract. The yield is expressed in pounds per quarter plot.

plots only. In 1913 and 1914 three cuttings were made. The first cutting was harvested in half plots. The second cutting of 1913 and the first and second cuttings of 1914 were harvested in quarter plots. The

third cutting of 1913 was lost because of a heavy wind which mixed the plot yields at harvest time, so that it was impossible to secure

III				II			
b		a		b		a	
85	85	130	120	130	150	140	165
105	100	105	120	135	150	140	185
100	80	105	110	120	150	170	165
105	110	95	130	165	155	150	170
100	100	105	130	120	140	145	185
100	105	100	125	120	175	195	155
90	100	100	120	155	155	115	200
90	100	105	120	85	155	145	170
120	95	90	120	115	140	170	165
85	95	75	110	155	130	105	155
75	95	85	105	85	130	125	240
60	110	90	100	120	140	160	135
75	100	75	140	95	120	120	130
55	100	75	140	120	130	125	165
75	95	85	125	120	130	140	145
85	100	60	115	125	120	140	160
85	105	100	105	120	135	135	150
115	100	65	115	115	140	155	130
115	125	85	125	150	125	140	130
85	135	95	120	135	135	135	135
105	120	105	105	130	140	165	145
100	115	125	135	140	160	170	140
100	115	140	120	135	120	115	120

FIG. 4.—Diagram showing yield of alfalfa in first cutting, 1914, on the Huntley experimental tract. The yield is expressed in pounds per quarter plot.

accurate weights on any of the plots. The third cutting for 1914 was harvested in subplots one-third the size of the original plots.

The actual yield of these subdivisions is indicated in figure 2¹ for the first cutting and figure 3 for the second cutting in 1913 and in figure 4

¹ Diagrams are set in type instead of being drawn to scale.

for the first cutting, figure 5 for the second cutting, and figure 6 for the third cutting in 1914.

III				II			
b		a		b		a	
100	110	135	125	120	145	145	140
80	85	110	120	130	145	175	155
70	110	140	115	170	155	195	170
70	140	115	125	160	190	145	165
85	125	85	125	180	190	155	175
55	125	95	100	190	175	185	185
65	105	115	115	225	155	200	195
65	110	95	110	190	190	180	165
70	105	100	135	140	155	155	165
110	120	60	100	110	120	100	175
100	110	85	125	95	125	70	140
95	120	120	95	75	100	145	105
110	135	125	135	100	75	125	145
130	120	95	150	135	85	90	170
115	115	100	140	115	125	105	170
130	130	80	115	95	110	95	140
135	115	65	110	110	85	90	150
110	115	80	120	120	130	95	180
145	160	75	135	120	125	105	140
140	135	80	125	105	145	155	100
135	135	90	120	115	155	140	125
120	155	110	130	130	130	135	130
90	160	110	115	120	130	120	75

FIG. 5.—Diagram showing yield of alfalfa in second cutting, 1914, on the Huntley experimental tract. The yield is expressed in pounds per quarter plot.

For the yield of alfalfa on quarter plots for the second cutting in 1913 and the first and second cuttings for 1914 and in third plots for the third cutting for 1914 the correlations are

1913, second cutting, $r=0.182 \pm 0.048$, $r/E_r= 3.79$.

1914, first cutting, $r=0.432\pm0.040, r/E_r=10.7$.
1914, second cutting, $r= .449\pm .040, r/E_r=11.3$.
1914, third cutting, $r= .311\pm .052, r/E_r= 5.99$.

III			II		
x	y	z	x	y	z
230	190	225	160	240	180
220	170	130	220	220	165
215	150	130	200	205	190
175	150	115	205	190	215
175	155	125	205	220	170
155	155	105	175	160	175
190	130	125	160	175	165
155	145	115	170	165	165
170	105	110	160	155	160
140	120	100	150	120	180
155	90	140	95	160	145
125	125	120	125	165	155
210	100	125	145	160	150
175	140	110	180	165	140
155	145	155	180	195	165
140	115	155	165	185	125
150	125	155	170	170	120
115	120	150	170	150	135
160	150	165	150	165	150
140	165	140	150	165	160
155	155	155	165	195	150
150	175	170	175	160	185
185	150	140	90	155	135

FIG. 6.—Diagram showing yield of alfalfa in third cutting, 1914, on the Huntley experimental tract.
The yield is expressed in pounds per third plot.

It will be noted that the results are in very close agreement indeed for 1914. The second cutting for 1913 differs significantly from the others, but no explanation can be suggested.

Grouping all yields in two comparable subplots, we find

1913, first cutting, $r = 0.407 \pm 0.059$, $r/E_r = 6.93$.

1913, second cutting, $r = .343 \pm .062$, $r/E_r = 5.52$.

1914, first cutting, $r = .602 \pm .045$, $r/E_r = 13.4$.

1914, second cutting, $r = .657 \pm .040$, $r/E_r = 16.4$.

We note that all the correlations are higher for a 2-fold division than for a 4-fold division. The coefficients for the second cutting of 1913 are again lower than the other values.

The foregoing results are based upon weightings of single cuttings only. It is now desirable to determine the correlations for yield of first and second cuttings combined.

If the combined yield be considered in quarter plots as ultimate units in 1914 we find

$$r = 0.517 \pm 0.036, r/E_r = 14.2.$$

Combining to obtain total yield in half plots in both 1913 and 1914, we have the following correlations between the yields of the two half plots:

For 1913, $r = 0.387 \pm 0.060$, $r/E_r = 6.46$.

For 1914, $r = .709 \pm .035$, $r/E_r = 20.2$.

5.—STRAW AND GRAIN IN WHEAT

The data of the Rothamsted wheat plots,¹ analyzed in an earlier paper (5, p. 436-440, 443-444), show the following correlations when the 500 plots are grouped in 2 by 2 fold manner for the first 22 files and in a 2 by 3 fold manner for the twenty-third to the twenty-fifth file:

For yield of grain, $r = 0.336 \pm 0.027$, $r/E_r = 12.5$.

For yield of straw, $r = .483 \pm .023$, $r/E_r = 20.9$.

6.—STRAW AND GRAIN IN RAGI, ELEUSINE CORACANA

Lehmann (12) has given a series of data derived from the yields of grain and straw of ragi cultivated on the dry-land tract of the Experimental Farm at Hebbel, near Bangalore, Mysore State. The plots used were of 1/10-acre area.

The land was previously owned by several raiyats who have naturally treated it somewhat differently in regard to manuring and cultivation. The various pieces used as garden lands are of course in much better condition than those used for ordinary dry crops. This causes considerable temporary differences to exist in some of the plots in addition to probably slight permanent differences. (12, 6th Rpt., p. 2.)

From these conditions one would expect a high degree of heterogeneity in the series of plots. The data permit the testing of the possibility of a decrease in heterogeneity due to uniformity of crop and treatment for three years.

¹ For data see Mercer and Hall (15, p. 119); also Map B of Harris (5).

These data are, furthermore, of particular interest since they consist of the records of yields for three successive years of the same crop on a series of unirrigated plots in a region where crop production is subject to many uncertainties because of inadequate rainfall.

Fortunately for our present purposes the meteorological conditions during the three years covered by this experiment were very different from year to year. The values of the most significant factor, the July to October rainfall, are given in Table I. This shows that the rainfall in 1906 was practically twice as heavy as in either of the other two years.¹

TABLE I.—Rainfall at Hebbel, near Bangalore, Mysore State, India

Month.	1905	1906	1907	Average of 10 years.
	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>	<i>Inches.</i>
July.....	1. 77	7. 09	4. 17	3. 04
August.....	6. 75	9. 98	1. 50	4. 32
September.....	1. 47	5. 50	5. 66	8. 14
October.....	5. 76	8. 51	. 81	5. 97
Total.....	15. 75	31. 08	12. 14	21. 47

Maps of the fields are given in the sixth annual report for 1904-1905. Further descriptive detail is given in the seventh, eighth, and ninth reports for 1905-1908. The yield of grain and straw in plots of 1/10 acre grown in 1905 is given in the seventh report. The eighth report gives detail of the crop of 1906 but does not contain the yields, which are summarized for the years 1905, 1906, and 1907 in Tables I and II of the ninth report.

Unfortunately the yields of a considerable number of the plots have had to be omitted from maps I and II of Lehmann's report. In combining in a 2 by 2 fold manner it is necessary either to disregard all combination plots in which there are not four ultimate plots or to weight properly in using those containing 2 or 3 plots only. The course followed has been to group the plots by fours and to determine the correlation by the formulae for a variable number of plots when all of the ultimate plots were not planted.

The following table shows the correlation between the yield of grain, of straw, and of grain and straw:

	1905	1906	1907
Grain.....	0. 735±0. 031	0. 138±0. 065	0. 716±0. 032
Straw.....	. 424± . 055	. 164± . 065	. 573± . 045
Total yield.....	. 415± . 055	. 145± . 065	. 636± . 040

¹ A discussion of the growth of these crops in relation to the distribution of the rainfall appears in Lehmann's ninth report (12, p. 2-7).

The results are of unusual interest. In 1905 and 1907 the correlation between yields of grain are unusually high, falling only slightly below three-fourths of perfect correlation. The correlations for yields of straw and for both grain and straw are of medium value in those two years. In 1906, however, the correlations for all the characters are of a very low order; and any one of them taken alone might not be considered significant in comparison with its probable error, which has been calculated on the basis of 103 plots, the number actually involved in the calculations.

Apparently the unusual moisture conditions of 1906 tended to obliterate the differences in the field to which the individuality of adjoining plots was due.

That the unusual weather had a profound influence on the yield of the plots is shown by Table II, in which the means, standard deviations, and coefficients of variation for the yield of the individual plots are set forth.¹

TABLE II.—Means, standard deviations, and coefficients of variation for the yield of ragi at Hebbel, near Bangalore, Mysore State, India

[Yield expressed in pounds per 1/10-acre plot]

Year.	Grain.			Straw.			Total yield.		
	Mean.	Stand-ard devi-ation.	Coeffi-cient of vari-ation.	Mean.	Stand-ard devi-ation.	Coeffi-cient of vari-ation.	Mean.	Stand-ard devi-ation.	Coeffi-cient of vari-ation.
1905.....	192.8	31.5	16.3	360.8	148.8	41.2	553.5	190.3	34.4
1906.....	136.6	47.1	34.5	191.6	82.0	42.8	328.1	127.4	38.8
1907.....	165.0	48.3	29.3	295.4	80.2	27.1	460.4	126.9	27.6

The means show that yield of both grain and straw was much lower in the abnormally wet year than in either of the others. The standard deviations are of course largely influenced by the actual magnitudes of the yields and are, in consequence, difficult of interpretation. The relative variabilities, as measured by the coefficients of variation, are more orderly. They show that for grain, straw, and total yield the variability of the individual plot yields is greater in the wet year.

Thus the influence of the wet season has not been to make the yield of all the plots alike. It has tended to decrease yield and to increase relative variability from plot to plot. But at the same time it has tended to screen certain factors which in drier years have a marked influence on the individuality of the plots.

Further analysis is not desirable without more detailed information concerning the plots. From the information at hand it seems quite

¹ These constants are obtained by weighting in an (n-1)-fold manner, since this was the method followed in obtaining the constants for the heterogeneity coefficient.

clear that the innate differences in different parts of the field do not in some seasons exert their full influence upon crop yield because of the weight of other factors. The practical conclusion to be drawn from this result is that an experimental field which might be demonstrated to be sensibly uniform for one crop plant or for one season might not prove to be so for another crop or in a different season.

7.—KHERSON OATS

Kiesselbach (10, 11) has given records of yield for 207 1/30-acre plots of Kherson oats. He says:

These plats were planted . . . upon a seemingly uniform field for the purpose of studying variation in plat yield as a source of experimental error. The entire field had been cropped uniformly to silage corn for a period of eight years. It had been plowed each year and was also plowed in preparation for the oats in 1916. The oats were drilled during two successive days in plats 16 rods by 66 inches . . . The plats were separated by a space of 16 inches between outside drill rows. A wide discard border of oats was grown around the outer edge of the field, so that all plats should have a similar exposure.

Love (13) has shown the existence of heterogeneity in this field.

Grouping the entries of Kiesselbach's Table 27 in a 3 by 1 fold manner the heterogeneity coefficient is found to be

$$r = 0.495 \pm 0.035, r/E_r = 14.$$

For data on a second test of the influence of heterogeneity on the yields of experimental plantings of oats we turn to a small experiment by Montgomery (17), who has given the yields of thrashed grain in grams from 100 consecutive rows of Kherson oats (17, p. 35, Table XIII) each 12.5 feet in length.

The plat chosen for this test was quite uniform and the appearance of the plat at harvest was very satisfactory.

Combining by twos, we find for the correlation between adjacent rows

$$r = 0.339 \pm 0.060, r/E_r = 5.65.$$

8.—GRAIN AND NITROGEN CONTENT IN WHEAT

Montgomery (17, p. 37, fig. 10) has given the yield of grain in grams on 224 blocks each 5.5 feet square. Combining in a 2 by 2 fold manner we deduce

$$r = 0.391 \pm 0.038, r/E_r = 10.2.$$

Again, Montgomery (17, p. 21-22, fig. 7) has given the values of nitrogen content from 224 Turkey wheat plots of the same size. These values are quoted in figure 1 of this paper. The correlation between the plots is found to be

$$r = 0.020 \pm 0.045, r/E_r = 0.44.$$

Finally, Montgomery (16) has given data for both yield of grain and nitrogen content on 224 plots of wheat grown at the University of Nebraska in 1911. The plot (77 by 88 feet) had been sown continuously to Turkey winter wheat for three years.

The plat was of about average uniformity and fertility.

When grouped in a 2 by 2 fold manner these plots of wheat have been shown (5, p. 440-441, map C) to give the following correlations:

For yield of grain,
$$r=0.603\pm0.029, r/E_r=21.$$

For percentage of nitrogen,
$$r=.115\pm.044, r/E_r=2.59.$$

Yield of grain per plot is clearly influenced by irregularities of the experimental field, notwithstanding the fact that the plots are only 5.5 by 5.5 feet in area. The correlation for percentage of nitrogen is not certainly significant.

9.—HOPS

Stockberger (20) has given a series of yields for 30 rows of hops which he believes to be quite typical of many thousands of acres in the Sacramento Valley in California. The yields of these rows cover the period of 1909 to 1914. Combining the rows by twos and determining the correlation between the yield of the adjacent rows of the 15 pairs for each of the years, we obtain the following constants:

Year.	Correlation.	r/E_r .
1909.....	0.444 ± 0.099	4.50
1910.....	$.695\pm.064$	10.91
1911.....	$.061\pm.123$	8.50
1912.....	$.326\pm.110$	2.97
1913.....	$.606\pm.078$	7.79
1914.....	$.386\pm.105$	3.69
Average.....	.419	5.06

Without exception the coefficients are positive in sign. In general they are fairly large and indicate a substantial degree of heterogeneity in this limited area. Probably the heterogeneity would have been shown to be greater had it been possible to work with yields from the sections of the long rows instead of with the rows as a whole.

10.—UNHUSKED RICE

Coombs and Grantham (2) give the yield in gantangs of a series of 54 square plots ½ by ½ chain in dimension.

These plots are arranged in 18 ranks and 3 files. They were harvested from a field of standing rice on which—

the crop was extremely regular, as judged before the cutting, and it had not been subjected to any attack of borer or any devastation of rats or birds.

The yields of the original plots are shown in figure 7. These may be combined in a 2 by 1 fold manner to give a correlation of

$r=0.344\pm0.081, r/E_r=4.25.$

These rice yields taken from a field described as “extremely regular” show that as a matter of fact the field is heterogeneous and that this irregularity influences in a measurable degree the yields of the plots.

13.6	12.0	11.4
14.6	14.0	12.2
14.8	14.4	12.0
13.0	12.4	12.8
15.0	12.0	12.0
13.4	13.8	14.0
14.2	12.2	13.0
14.0	12.0	12.8
14.0	12.0	13.4
14.0	14.0	12.4
15.0	14.0	12.6
14.8	14.0	12.4
14.0	14.0	12.0
14.4	13.6	12.4
12.6	13.0	12.0
12.2	14.0	12.8
11.6	12.0	11.8
12.4	14.0	12.4

FIG. 7.—Diagram showing yield of unhusked rice on Coombs and Grantham's 54 plots ½ by ½ chain square. The yield is expressed in gantangs per plot.

11.—EAR CORN

Smith (18) has published a series of corn yields for three years on plots of 1/10 acre. The yields are given in his original paper. He has kindly supplied the map showing the relative positions of these plots, which are arranged thus:

101, 201, . . . , 601
102, 202, . . . , 602
 , , ,
 , , ,
 , , ,
120, 220, . . . , 620

Combining yields in a 2 by 1 fold manner, we find for the correlation between the yields of adjacent $\frac{1}{10}$ -acre plots

For 1895, $r = +0.830 \pm 0.019, r/E_r = 43.4$.

For 1896, $r = +.815 \pm .021, r/E_r = 39.6$.

For 1897, $r = +.606 \pm .039, r/E_r = 15.5$.

It is evident that the field was rather highly heterogeneous.

III				II			
b		a		b		a	
133	132	138	142	136	132	148	140
141	141	132	138	145	135	162	156
135	109	125	135	133	116	147	130
132	153	131	131	130	123	155	150
132	137	135	140	137	112	131	129
135	132	135	131	134	126	126	135
131	128	121	125	126	115	122	136
135	125	128	131	121	115	129	137
133	125	125	130	131	124	129	131
137	124	117	131	127	125	129	132
130	117	119	127	132	129	122	141
134	122	115	125	133	123	119	132
129	122	120	132	130	125	136	137
123	118	125	130	124	124	123	136
129	126	134	129	122	126	127	136
134	124	120	121	126	130	132	136
128	125	115	115	122	123	140	135
128	121	110	110	116	115	125	123
127	124	119	107	114	116	110	115
134	112	121	123	122	126	116	125
145	148	133	125	132	127	126	134
149	154	165	160	162	144	137	130
168	169	165	152	158	169	143	108

FIG. 8.—Diagram showing yield of ear corn, 1915, on the Huntley experimental tract. The yield is expressed in pounds per quarter plot.

For a second test of the influence of field heterogeneity on the yield of ear corn we turn to the Huntley data.

III				II			
b		a		b		a	
78	94	104	128	110	121	132	150
73	81	104	118	116	116	140	142
66	77	84	110	113	102	128	138
66	73	80	99	115	113	128	139
77	79	79	103	116	118	126	127
71	73	86	82	100	110	108	132
76	59	86	90	110	117	111	151
94	65	86	100	102	105	118	116
98	75	80	100	111	101	104	118
88	76	74	99	108	92	102	113
91	82	69	80	100	97	101	101
97	87	83	90	103	92	88	96
75	81	80	107	96	78	96	106
67	76	73	117	95	70	90	117
98	85	74	103	98	84	100	116
111	88	76	97	97	92	110	110
108	88	73	84	84	86	104	115
115	97	66	89	100	87	98	123
104	120	86	100	94	94	97	119
110	106	92	99	96	100	83	104
118	110	100	98	114	108	113	120
108	100	105	110	93	99	117	104
108	98	95	100	103	99	114	98

FIG. 9.—Diagram showing yield of ear corn, 1916, on the Huntley experimental tract. The yield is expressed in pounds per quarter plot.

In 1915 and 1916 corn was grown on the Huntley experimental plots, described above, and was harvested in quarter plots. The yields for the two series are shown in figure 8 for 1915 and in figure 9 for 1916. These records are of special interest in view of the fact that these are irrigated

fields, whereas the data provided by Smith are based on corn grown without irrigation.

Retaining the original division into quarter plots, we deduce for the correlation between the subplots

For 1915, $r = 0.498 \pm 0.037$, $r/E_r = 13.4$.

For 1916, $r = .436 \pm .040$, $r/E_r = 10.8$.

The results for the two years can not, with due regard to their probable errors, be considered to differ significantly. They indicate a degree of heterogeneity in these Huntley plots quite comparable with that of fields planted to various crops by other observers.

If the quarter plots be combined by adjacent twos and the correlation between the half plots be determined, we find

For 1915, $r = 0.494 \pm 0.053$, $r/E_r = 9.29$.

For 1916, $r = .0431 \pm .057$, $r/E_r = 7.53$.

The measure of heterogeneity has been only slightly lowered by dividing the plots into halves instead of into quarters.

INFLUENCE OF SUBSTRATUM HETEROGENEITY ON YIELD OF ORCHARD CROPS

In the preceding illustrations the crops considered have been herbaceous plants which are generally fairly superficial in their relation to the soil and most of which complete their development in one or two seasons. It seems of particular interest to extend the studies, as Batchelor and Reed (1) have done, to the yield of large individual plants, such as orchard trees.

For the purpose we employ the splendid series of data of Batchelor and Reed. They say of their various groves (1, p. 251):

The fruit plantations herein discussed, to judge by the surface soil, size, and condition of the trees, as well as their apparent fruitfulness, appeal to the observer as uncommonly uniform. All the orchards studied are situated in semiarid regions and are artificially irrigated during the summer months. This fact is believed to be a distinct advantage for the purpose of reducing the variability of one year's yield compared with another, since it insures a fairly uniform water supply for the soil and reduces one of the variants inevitable in nonirrigated localities.

In the case of the Arlington navel oranges grouped in 8-tree plots as the ultimate unit the authors (1, p. 264) report a correlation between plots of $r = 0.533 \pm 0.085$ when the plots are combined by fours.

It has seemed desirable to test the homogeneity of the soil in each of the orchards studied by them. In determining the following coefficients the individual tree has in each case been the ultimate unit.¹

Consider first the relationship between the yields of adjacent trees of two navel orange groves.

¹ Yields are reported in pounds per tree of ungraded product.

Grouping the yield of the 1,000 trees at Arlington, shown in figure 1 of Batchelor and Reed, in a 2 by 2 fold manner we find

$$r=0.517 \pm 0.016, r/E_r=33.1.$$

A navel orange grove of 495 trees at Antelope Heights, mapped as figure 2 by Batchelor and Reed, when combined in a 3 by 3 fold manner gives

$$r=0.375 \pm 0.026, r/E_r=14.4.$$

Grouping the 240 Valencia orange trees of the grove shown in figure 3 of Batchelor and Reed in a 2 by 2 fold manner, we find for the correlation between yields

$$r=0.306 \pm 0.039, r/E_r=7.75.$$

For the yield in pounds per tree of Eureka lemons as shown in figure 4 of the authors cited, we find for a 2 by 2 fold grouping

$$r=0.448 \pm 0.028, r/E_r=15.8.$$

This last result is of particular interest, since Batchelor and Reed say of this plantation—

This grove presents the most uniform appearance of any under consideration. The land is practically level, and the soil is apparently uniform in texture. The records show a grouping of several low-yielding trees; yet a field observation gives one the impression that the grove as a whole is remarkably uniform.

Notwithstanding this apparent homogeneity there is a heterogeneity coefficient of over 0.4.

Taking the yields of seedling walnuts in pounds per tree as given in figure 5 of Batchelor and Reed and grouping in a 2 by 2 fold manner, we find

$$r=0.232 \pm 0.038, r/E_r=6.09.$$

Finally, if the yields in pounds per tree of the Jonathan apple trees mapped by Batchelor and Reed in their figure 6 be treated in a 2 by 2 fold grouping, the coefficient is

$$r=0.214 \pm 0.043, r/E_r=4.97.$$

Without exception these groves show material values of the heterogeneity coefficients which are statistically significant in comparison with their probable errors throughout.

PHYSICAL, AND CHEMICAL, BASIS OF THE HETEROGENEITY OF EXPERIMENTAL FIELDS

In foregoing sections it has been shown that when tracts of land are judged by their capacity for crop production the yields are such as to indicate that heterogeneity is a practically universal characteristic of the

fields which may be used for fertilizer tests, variety trials, or any other experimental purpose involving plot yields. In the vast majority of cases the heterogeneity is so great as to leave open to question conclusions drawn from experiments not carried out with all biological precautions and interpreted with due regard to probable errors.

While the actual demonstration of differences in crop yields from one portion of the field to another is the result of final importance from the agronomic standpoint, and while it furnishes all but conclusive evidence that this heterogeneity in yield is due to irregularities in the soil itself, it seems desirable to show that such heterogeneity does actually obtain in the physical and chemical properties of the soil which are determining factors in plant growth.

The desirability of determining the extent to which heterogeneity, in the sense to which the term is used here, obtains in the physical and chemical properties of the soil of experimental fields is emphasized by the following sentences from one of the pioneer papers (21) on the variability of soil samples.

A number of papers have appeared dealing with the variation in the weight of the crop produced over different parts of an apparently uniform field. Such variations reflect the variability of the soil, serving simply as a substratum for the growth of plants, but it is evident that the variations between such measurements as those given do not depend upon the soil as the only variable factor.

At the outset we must recognize that many factors may determine differences in yield. Even if one could secure a tract initially uniform in soil and exposure it is not always possible to be sure that it has all been in the same crop in preceding years. Previous cultures may influence tilth and soil composition by organic remains, by infection with disease-producing organisms, or by differences in the demand of various crops for certain of the plant foods.¹ Such sources of heterogeneity are not readily detected by the eye or by physical or chemical analysis. Even if the experimenter secures a field of sensibly uniform texture, chemical composition, and previous cultural treatment, the uniformity may be readily destroyed in planting or tillage. Rain may interrupt the ploughing, thus exposing the soil of the different portions of the field to air and light for different lengths of time and affecting the physical condition very profoundly. Such sources of error are particularly great in the planting of large experiments. Thus the sources of field heterogeneity can never be fully determined in any case, although individual factors may be demonstrated.

To determine whether an experimental field is heterogeneous with respect to physical or chemical factors, actual measurements of these factors should be made over the field and the heterogeneity coefficient applied. As a first illustration we take a series of soil-moisture

¹ These are factors of particular importance in rotation experiments.

determinations uniformly distributed over a plot on a field at the San Antonio Experimental Farm of the Office of Western Irrigation Agriculture.

Hastings (6) has given a condensed account of the soil conditions of the San Antonio region. A map of the experimental farm by Hastings (7, *p. 2*) shows the location of field C₃ in which this plot of borings was located¹ and gives meteorological conditions prevailing in 1915, the year in which the borings were made.

Mr. C. S. Scofield kindly informs me that field C₃ had been uniformly treated for some time previously and was in apparently uniform condition. It is nearly level but with a gradual slope to the south and east.

The soil has the superficial appearance of uniformity, but we know from experience that the subsoil, which is usually characterized by a high lime content, is in some

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34	35	36	37	38	39
40	41	42	43	44	45	46	47	48	49	50	51	52
53	54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77	78
79	80	81	82	83	84	85	86	87	88	89	90	91
92	93	94	95	96	97	98	99	100				

FIG. 10.—Diagram showing location of sample areas examined for soil moisture in a field at the San Antonio Experimental Farm.

places much closer to the surface than in others. However, from a general agronomic standpoint, this field would be regarded as extremely uniform, and observation of it during the growing season would tend to confirm this view.

Borings were made 6 feet in depth and were sampled at every foot.² Figure 10 shows the form of this field.

In order to reduce the 100 sample areas to 2 by 2 fold combinations we have discarded the right file and a portion of one rank, retaining only those which can be grouped into fours as indicated by the cross lines. The percentages of moisture content of these 100 sample areas appear in Table III.³

¹ The northern border of the sampled area is a line 60 feet south of the north line of the field and parallel to it.

² The samples were all taken between March 31 and April 9. During this period there was no rain. Between March 15 and April 10 there were only two rains, one on March 17 of 0.2 inch, the other on March 29 of 0.01 inch. Neither of these was sufficient to affect the soil moisture conditions, since in this region a precipitation of less than 0.25 inch scarcely penetrates the surface-soil mulch. Thus moisture changes during the course of the work can hardly influence the results.

³ The 12 sample areas which were omitted because of impossibility of combining by fours are starred (*).

TABLE III.—*Moisture content of 100 sample areas of a field at the San Antonio Experimental Farm*

[Expressed in percentages]

Sample area No.	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.
1	20.2	19.1	17.5	13.1	9.7	8.9
2	23.7	21.6	19.8	16.8	15.0	15.9
3	20.9	20.5	19.4	16.1	15.0	15.1
4	21.5	20.3	18.3	16.0	15.4	14.2
5	23.3	22.4	20.6	19.3	16.1	15.8
6	25.0	24.1	19.7	17.6	16.5	15.3
7	22.8	23.0	20.8	17.0	14.8	14.8
8	24.6	24.3	20.7	18.5	15.8	14.8
9	25.6	25.3	25.3	25.5	23.7	18.7
10	22.9	25.8	26.0	26.2	23.5	18.6
11	28.0	30.4	30.6	29.8	26.8	21.5
12	25.2	25.7	24.5	26.8	24.0	21.2
13*	22.1	22.0	20.1	20.1	16.0	14.9
14	20.2	19.7	17.0	14.5	11.4	9.1
15	22.1	21.3	18.1	14.6	13.8	12.7
16	25.1	21.2	20.0	16.3	15.5	14.1
17	21.8	21.0	19.2	16.6	15.1	14.9
18	23.4	22.4	20.0	16.1	15.7	15.4
19	20.5	20.8	19.6	15.6	13.5	12.5
20	24.0	22.0	19.5	15.1	11.5	9.4
21	20.4	20.7	18.7	13.0	8.1	8.2
22	24.3	24.0	21.0	23.7	21.3	15.2
23	21.3	22.2	21.8	21.7	20.5	16.7
24	24.3	25.7	24.0	22.4	18.3	14.9
25	23.6	23.2	24.4	24.5	22.2	18.4

determinations uniformly distributed over a plot on a field at the San Antonio Experimental Farm of the Office of Western Irrigation Agriculture.

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14	15	16	17	18	19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34	35	36	37	38	39
40	41	42	43	44	45	46	47	48	49	50	51	52
53	54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77	78
79	80	81	82	83	84	85	86	87	88	89	90	91
92	93	94	95	96	97	98	99	100				

FIG. 10.—Diagram showing location of sample areas examined for soil moisture in a field at the San Antonio Experimental Farm.

places much closer to the surface than in others. However, from a general agronomic standpoint, this field would be regarded as extremely uniform, and observation of it during the growing season would tend to confirm this view.

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TABLE III.—Moisture content of 100 sample areas of a field at the San Antonio Experimental Farm

[Expressed in percentages]

Sample area No.	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.
1	20.2	19.1	17.5	13.1	9.7	8.9
2	23.7	21.6	19.8	16.8	15.0	15.9
3	20.9	20.5	19.4	16.1	15.0	15.1
4	21.5	20.3	18.3	16.0	15.4	14.2
5	23.3	22.4	20.6	19.3	16.1	15.8
6	25.0	24.1	19.7	17.6	16.5	15.3
7	22.8	23.0	20.8	17.0	14.8	14.8
8	24.6	24.3	20.7	18.5	15.8	14.8
9	25.6	25.3	25.3	25.5	23.7	18.7
10	22.9	25.8	26.0	26.2	23.5	18.6
11	28.0	30.4	30.6	29.8	26.8	21.5
12	25.2	25.7	24.5	26.8	24.0	21.2
13*	22.1	22.0	20.1	20.1	16.0	14.9
14	20.2	19.7	17.0	14.5	11.4	9.1
15	22.1	21.3	18.1	14.6	13.8	12.7
16	25.1	21.2	20.0	16.3	15.5	14.1
17	21.8	21.0	19.2	16.6	15.1	14.9
18	23.4	22.4	20.0	16.1	15.7	15.4
19	20.5	20.8	19.6	15.6	13.5	12.5
20	24.0	22.0	19.5	15.1	11.5	9.4
21	20.4	20.7	18.7	13.0	8.1	8.2
22	24.3	24.0	21.0	23.7	21.3	15.2
23	21.3	22.2	21.8	21.7	20.5	16.7
24	24.3	25.7	24.0	22.4	18.3	14.9
25	23.6	23.2	24.4	24.5	22.2	18.4

TABLE III.—*Moisture content of 100 sample areas of a field at the San Antonio Experimental Farm—Continued*

[Expressed in percentages]

Sample area No.	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.
26*	24.2	23.7	23.0	20.7	19.1	17.8
27	21.1	19.7	18.7	14.7	14.5	17.7
28	21.2	19.6	18.4	17.6	15.2	15.0
29	21.2	20.5	19.6	18.9	17.5	17.1
30	22.9	22.0	19.9	17.5	15.0	14.8
31	21.0	20.7	19.6	16.2	14.0	16.4
32	23.4	21.6	19.3	18.6	16.8	15.9
33	22.2	21.8	20.1	16.6	14.0	14.1
34	23.9	22.7	20.4	17.0	14.6	14.2
35	21.6	20.9	19.2	16.8	15.3	16.3
36	21.4	21.6	20.6	20.0	18.4	16.8
37	25.3	25.6	25.6	24.9	22.2	17.9
38	26.7	29.2	27.0	25.9	23.0	19.1
39*	26.2	29.8	30.4	28.6	26.1	21.5
40	21.8	20.0	19.3	15.7	15.7	16.3
41	19.9	19.4	19.0	15.3	14.8	14.9
42	21.6	20.0	18.2	14.1	15.5	15.0
43	19.6	21.7	19.0	14.7	14.2	13.9
44	21.6	21.7	19.4	15.6	15.3	15.4
45	21.6	20.5	19.3	16.3	7.5	14.2
46	22.6	21.3	12.2	16.2	14.4	14.3
47	21.0	22.0	19.3	15.9	14.7	15.0
48	22.0	21.5	19.8	19.8	14.7	15.2
49	22.7	22.1	20.0	19.5	16.2	16.2
50	21.9	23.3	21.0	19.1	16.2	16.5

TABLE III.—*Moisture content of 100 sample areas of a field at the San Antonio Experimental Farm—Continued*

[Expressed in percentages]

Sample area No.	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.
51	20.0	20.3	19.0	17.6	15.7	17.2
52*	29.6	28.4	27.3	22.0	13.2	16.2
53	20.6	19.8	18.5	15.7	15.9	15.6
54	21.2	20.7	18.8	15.1	14.3	14.5
55	19.3	20.0	18.9	16.3	14.1	14.9
56	21.2	20.8	18.9	16.3	14.1	14.9
57	22.1	21.0	19.5	15.7	15.1	15.7
58	22.7	21.6	19.7	18.3	14.7	15.8
59	21.2	21.0	19.7	17.4	15.2	16.4
60	23.2	22.5	20.7	19.1	16.5	16.5
61	19.4	21.3	19.7	17.8	16.9	17.2
62	22.6	21.3	18.6	18.6	15.6	17.1
63	21.3	20.6	19.3	17.5	17.3	17.9
64	21.7	20.1	18.9	15.8	15.5	16.6
65*	22.5	21.0	20.2	16.7	17.0	20.8
66	20.6	21.2	17.9	17.1	15.8	15.0
67	18.9	19.2	18.2	15.0	14.4	15.0
68	23.4	14.5	19.0	17.7	15.5	15.5
69	21.2	20.4	18.8	17.0	14.0	13.9
70	21.4	20.1	18.4	17.0	15.8	16.0
71	21.0	21.1	18.9	15.6	14.5	15.3
72	22.8	21.4	20.0	16.5	15.5	14.3
73	21.9	21.6	20.2	16.2	14.4	17.9
74	22.8	21.8	20.3	18.0	15.5	17.9
75	21.3	22.8	22.1	21.6	17.7	15.1

TABLE III.—*Moisture content of 100 sample areas of a field at the San Antonio Experimental Farm—Continued*

[Expressed in percentages]

Sample area No.	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.
76	21.5	22.0	19.7	17.3	17.2	16.9
77	21.4	21.0	19.7	15.8	15.6	18.0
78*	22.4	21.0	19.2	17.0	16.2	15.6
79	18.5	18.8	18.4	17.0	15.2	15.1
80	20.3	19.6	18.5	15.2	15.0	15.8
81	20.3	20.2	18.8	16.5	14.6	15.5
82	21.5	21.7	18.6	15.8	14.8	14.1
83	20.0	20.4	18.7	15.7	16.3	15.4
84	20.3	20.0	18.9	17.5	14.7	14.7
85	22.4	21.8	21.4	17.1	15.9	14.8
86	23.2	22.0	19.6	16.0	15.7	15.0
87*	21.8	21.6	20.8	19.1	17.2	16.5
88*	23.7	21.8	20.2	16.4	16.9	16.4
89*	28.0	21.6	20.2	18.3	17.0	18.5
90*	23.2	21.7	19.1	16.3	16.3	16.6
91*	22.3	22.9	21.7	19.3	18.5	18.6
92	20.2	19.7	18.2	17.4	14.7	15.0
93	19.0	19.3	18.5	16.1	15.4	15.9
94	22.0	20.4	18.3	16.0	14.9	14.0
95	21.5	19.7	18.8	14.9	14.9	14.5
96	20.8	20.3	18.7	16.3	14.4	15.4
97	20.1	19.5	19.1	17.9	15.0	16.3
98	22.6	20.3	19.4	15.3	15.0	15.3
99	20.4	20.3	18.6	16.4	14.6	14.5
100*	22.6	21.6	19.4	17.5	16.3	15.0

To determine whether the distribution of soil moisture in these plots is such that it might bring about a correlation between the yields of adjacent plots due to heterogeneity in regard to this physical factor in

the field we have merely to determine the correlations between the percentages of water content of associated plots. These are

Depth.	Correlation.	r/E_r .
First foot.....	0.317 ± 0.065	4.9
Second foot.....	$.529 \pm .052$	10.2
Third foot.....	$.542 \pm .051$	10.7
Fourth foot.....	$.704 \pm .036$	19.4
Fifth foot.....	$.607 \pm .045$	13.4
Sixth foot.....	$.484 \pm .055$	8.8

The correlations are of a very substantial order, ranging from 0.317 to 0.704. Notwithstanding the fact that there are only 88 stations upon which the probable errors are based, the constants may in every case be considered significant in comparison with their probable errors.

Thus, notwithstanding the fact that we are dealing with a field only 150 by less than 264 feet,¹ there is a marked and statistically significant heterogeneity in respect to so important a factor in plant growth as soil moisture at each level in the upper 6 feet of soil.

This result seems of very real importance in its relation to the practical phases of plot-test work. It shows beyond all dispute that at least under soil conditions such as are found at the San Antonio Experimental Farm, substratum heterogeneity may be very great at levels of the soil which are ordinarily left entirely out of account in the selection of fields which are to be used for plot tests but which are not below the extensions of the roots of the deeper-penetrating crops and not too deep to serve as reserves of soil moisture for the higher layers of the soil in the case of crops which draw their water from more superficial levels.

It is of some interest to determine whether the correlations at one level in the field may be looked upon as sensibly higher than those at other levels. We have, therefore, determined the differences between the correlations at the different depths. These are given with their probable errors, and in relation to their probable errors, in Table IV.

In the table the positive signs indicate higher correlations at lower levels. Of the 10 possible comparisons between the correlations of the first 5 feet, all but one show greater heterogeneity at the lower levels. The sixth foot seems to be somewhat more homogeneous than the second to the fifth foot. A number of the differences are apparently significant in comparison with their probable errors. Thus there is apparently a real difference in the amount of heterogeneity of this field at different levels. Heterogeneity is least at the surface and greatest at a depth of 4 feet.

The significance of this result will perhaps be apparent at once. A field might be reasonably uniform for the surface foot of soil and hence

¹ The total length is 264 feet, but this is reduced by discarding the right file.

fairly well suited to the testing of shallow-rooted crops. Below this it might show a higher degree of heterogeneity. Possibly this heterogeneity of lower-lying strata is the explanation of the large correlations obtained for the yields of neighboring trees in groves planted on apparently uniform soil.

TABLE IV.—Differences and criteria of trustworthiness of differences in the correlation of adjacent plots in soil moisture determinations at various levels

Depth.	Second foot.		Third foot.		Fourth foot.		Fifth foot.		Sixth foot.	
	r.	r/Er.	r.	r/Er.	r.	r/Er.	r.	r/Er.	r.	r/Er.
First foot.	+0.212	2.56	+0.226	2.74	+0.387	5.22	+0.291	3.68	+0.167	1.97
	± .083		± .082		± .074		± .079		± .085	
Second foot.			+ .013	.18	+ .175	2.76	+ .078	1.14	— .045	.60
			± .073		± .063		± .069		± .076	
Third foot.					+ .161	2.58	+ .065	.96	— .059	.79
					± .062		± .068		± .074	
Fourth foot.							— .096	1.66	— .220	3.34
							± .058		± .066	
Fifth foot.									— .124	1.74
									± .071	

We can pursue this question of the relationship between the water content of the plots somewhat further. If the factors which determine the similarity in the moisture contents of the combination plots affect more than a single layer, we should expect a correlation between the contents of the first and second foot, and so on, in the same boring. The possible correlations have been worked out for the first foot and the remaining layers and are as follows:

Depth.	Correlation.	r/Er.
First and second feet.	+0.748 ± 0.032	23.59
First and third feet.	+ .669 ± .040	16.84
First and fourth feet.	+ .648 ± .042	15.53
First and fifth feet.	+ .578 ± .048	12.06
First and sixth feet.	+ .353 ± .063	5.62

There is a statistically significant and even high correlation between the water content of successive levels in the same boring.

When we turn to the problem of chemical heterogeneity, we find that while a number of soil chemists have noted the desirability of considering the variability of the soil in taking samples, the available data suitable for testing the degree of heterogeneity of experimental fields are not extensive.

Kaserer's series of determinations (9) is not sufficiently large or properly distributed over the field to make desirable an attempt to measure heterogeneity. Fortunately Waynick and Sharp (22) have given four excellent series, two for nitrogen and two for carbon, derived from two California fields.

Their samples were taken over a total area of a little more than 1.3 acres on two fields of very different character—a silty clay loam at Davis and a blow sand at Oakley.

The fields were both selected for their apparent uniformity, both being nearly level with no change in the soil mass from one part of the field to another great enough to be detected by the usual field methods. Both fields were practically free from vegetation when selected, and before the samplings were made in March, 1918, all extraneous material had been carefully removed.

Altogether they took 80 samples distributed at 30-foot intervals over the entire area. These samples were arranged in an 8 by 10 fold manner. The original data are given in their Tables 3 and 4. Arranging these in the order of the map of the borings given in their figure 1 and combining in a 2 by 2 fold manner, we derive the following heterogeneity coefficients:

For the silty clay loam at Davis—

For carbon, $r = 0.417 \pm 0.063$, $r/E_r = 6.67$.

For nitrogen, $r = .498 \pm .057$, $r/E_r = 8.75$.

For the blow sand at Oakley—

For carbon, $r = 0.317 \pm 0.068$, $r/E_r = 4.65$.

For nitrogen, $r = .230 \pm .072$, $r/E_r = 3.20$.

All these values are statistically significant in comparison with their probable errors. Although the total number of samples is rather small, they indicate in each case a distinct heterogeneity for these important constituents of the soil. Apparently the two fields differ in their heterogeneity, the coefficients for both carbon and nitrogen being distinctly lower on the blow sand at Oakley than on the silty clay loam at Davis. The average carbon content at Oakley is only 0.444 as compared with 1.109 at Davis, while the nitrogen at Oakley is 0.033 as compared with 0.101 at Davis. Probably greater heterogeneity would be expected on general physical considerations on the silt loam than on the blow sand.

The analysis may profitably be carried one step farther. If these fields are heterogeneous in respect to the soil constituents here under consideration, one might anticipate a correlation between the carbon and the nitrogen content of the samples distributed over these fields. The results are

For the Davis loam, $r_{nc} = 0.785 \pm 0.029$, $r/E_r = 27$.

For the Oakley blow sand, $r_{nc} = .744 \pm .034$, $r/E_r = 22$.

Both constants are large. They show that the field is not merely heterogeneous but that portions which are high in nitrogen are high also in carbon and vice versa.

Waynick (21) has given a series of 81 determinations of nitrification in samples of soil drawn from a field on the University of California farm at Davis.

The field had been planted to corn in 1914, to Sudan grass in 1915, and to grain sorghum in 1916. In 1917 it had lain fallow and was without vegetation when the samples were taken October 20.

The particular area chosen was apparently as uniform as one could well find, being level, of uniform texture and color, and free from small local depression of any kind.

These samples were taken on eight radii of a circle 100 feet in diameter. The samples were separated by a radial distance of 5 feet. Disregarding the one central sample, we may group the remainder by twos in order to determine whether there is a correlation between adjacent samples. The coefficients thus obtained will, of course, not be comparable with those deduced for cases in which the yields or soil samples were uniformly distributed over the field. They will, however, serve to indicate whether or not this field is heterogeneous in the sense that differences prevailed sufficiently large to influence the properties of adjacent samples in a manner to make them more similar than pairs of samples taken at random over the field. His samples were drawn in two series—the first from the superficial 6 inches, the second from the deeper-lying level, 6 to 24 inches.

Waynick's Table 1 gives the residual nitrate in soil as sampled. From it we deduce

For the upper 6 inches, $r = 0.404 \pm 0.063$, $r/E_r = 6.4$.

For the subsoil, $r = .596 \pm .049$, $r/E_r = 12.2$.

Table 2 gives the nitrate produced from the soil's own nitrogen after 28 days' incubation. We deduce

For the upper 6 inches, $r = 0.065 \pm 0.075$, $r/E_r = 0.86$.

For the subsoil, $r = .059 \pm .075$, $r/E_r = .79$.

Table 3 shows the nitrate produced from 0.2 gm. of ammonium sulphate in 100 gm. of soil. The correlation coefficients are

For the upper 6 inches, $r = 0.298 \pm 0.069$, $r/E_r = 4.34$.

For the subsoil, $r = .351 \pm .066$, $r/E_r = 5.31$.

Finally, Table 4 shows the nitrate produced from 0.2 gm. of blood in 100 gm. of soil. The results in this case are

For the upper 6 inches, $r = 0.120 \pm 0.074$, $r/E_r = 1.62$.

For the subsoil, $r = .297 \pm .069$, $r/E_r = 4.32$.

The coefficients show that for both the upper and lower soil layers there is a correlation of about medium value between adjacent samples for the residual nitrate in the soil. These coefficients are unquestionably significant in comparison with their probable errors.

While the coefficients for nitrogen produced from soil nitrogen after incubation are both positive in sign, neither can be considered statistically trustworthy in comparison with its probable error. When nitrogen is added to the soil, in the form of either ammonium sulphate or of blood, the correlations between the nitrogen produced on incubation are larger. All are positive in sign, and three of the four may be reasonably considered statistically significant.

Thus it is clear that this plot, only 100 feet in diameter, shows distinct heterogeneity in residual nitrate and in the amount of nitrification occurring on incubation after the addition of nitrogen.

SUMMARY AND CONCLUSIONS

The purpose of this paper, which is one of a series on the statistical phases of the problem of plot tests, is to show the extent to which the heterogeneity of experimental fields may influence plot yields.

By heterogeneity we understand differences in capacity for crop production throughout the field of such a magnitude as to influence in like manner, but not necessarily to like degree, the yield of adjacent small plots. Thus, variability of plot yields does not necessarily indicate the heterogeneity of the fields upon which tests are made but may be due to other factors.

Heterogeneity is measured by a coefficient which shows the degree of correlations between the yields of associated ultimate plots, grouped in combination plots.

This coefficient has been determined for a relatively large series of experimental fields widely distributed throughout the world and planted to a considerable variety of crops, for which a number of different kinds of yields have been measured. The results show that in every field the irregularities of the substratum have been sufficient to influence, and often profoundly, the experimental results.

It might be objected that by chance, or otherwise, the illustrations are not typical of what ordinarily occurs in plot cultures. But the series considered practically exhaust the available data for such purposes. Furthermore the records are in large part drawn from the writings of those who are recognized authorities in agricultural experimentation and who have given their assurance of the suitability of the fields upon which the tests were made.

For example, Mercer and Hall (15) state the purpose of their research to be—

to estimate the variations in the yield of various sized plots of ordinary field crops which had been subjected to no special treatment and appeared to the eye sensibly uniform.

Their mangolds—

looked a uniform and fairly heavy crop for the season and soil,
while in their wheat field—
a very uniform area was selected.

The data of Larsen were drawn from an experiment—
auf einer dem Auge sehr gleichmassig erscheinenden, 3 Jahre alten Timotheegraswiese.

Montgomery's data were secured from a plot of land only 77 by 88 feet in size, which had been sown continuously to Turkey wheat for three years—

and was of about average uniformity and fertility.

Coombs and Grantham selected a field on which—
the crop was extremely regular as judged before the cutting and it had not been subjected to any attack of borer or any devastation of rats or birds.

Lyon's potato field was selected from—
a piece of apparently uniform land.

Mr. C. S. Scofield kindly informs us that the Huntley tract was selected for apparent uniformity and that prior to the calculation of the constants presented in this paper there was no reason, from general observation, to suspect irregularities in the field. Batchelor and Reed have assured me that their orchards are to all appearances uncommonly uniform. Kiesselbach emphasizes the apparent uniformity of his oat field.

Nothing could more emphasize the need of a scientific criterion for substratum homogeneity than the fact that correlations between the yields of adjacent plots ranging from $r = +0.020$ to $r = +0.830$ can be deduced from the data of fields which have passed the trained eyes of agricultural experimenters as satisfactorily uniform.

A second phase of this investigation has been to ascertain whether the physical or chemical requisites for plant growth are so distributed over experimental fields that they may be reasonably looked upon as the source of the demonstrated heterogeneity in yield.

The heterogeneity coefficients for percentage of water content for the upper 6 feet on the Experimental Farm of the Office of Western Irrigation Agriculture at San Antonio, Tex., range from $+0.32$ to $+0.70$ and are statistically significant for each of the 6 upper feet of soil. Heterogeneity is least at the surface and greatest at a depth of 4 feet. The surface layer of soil might, therefore, be apparently uniform in water content while underlying layers might differ greatly from one part of the field to another. This may be the explanation of the correlation between the yields of adjacent trees in groves planted in an apparently uniform locality.

Analysis of the data of Waynick and Sharp shows that there is a correlation of from $+0.23$ to $+0.50$ between adjacent borings for so important soil constituents as nitrogen and carbon. The correlation

between nitrogen content and carbon content of samples from two different soils is of the order $+ 0.75$.

It is interesting to note that these coefficients for water content and for chemical composition of the soil are of about the same order as those found for crop yields. While these results do not prove that the heterogeneity of experimental fields in their capacity for crop production is directly due to these and other physical and chemical factors, there can be little doubt that this is actually the case.

The references here made to the existence of significant heterogeneity in fields passed by agricultural experts as satisfactorily uniform must not be interpreted as a criticism of the work of these investigators. There is, indeed, every evidence of care and thoroughness. The result merely illustrates the inadequacy of personal judgment concerning the uniformity in physical characters or in crop-producing capacity of fields under consideration for experimental work.

The demonstration that the fields upon which plot tests have been carried out in the past are practically without exception so heterogeneous as to influence profoundly the yields of the plots emphasizes the necessity for greater care in agronomic technic and more extensive use of the statistical method in the analysis of the data of plot trials if they are to be of value in the solution of agricultural problems.

To other phases of the problem we shall return in subsequent papers.

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TRANSMISSION OF THE MOSAIC DISEASE OF IRISH POTATOES¹

By E. S. SCHULTZ, *Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture, and*
DONALD FOLSOM, *Assistant Plant Pathologist, Maine Agricultural Experiment Station*

INTRODUCTION

In a previous publication² evidence was presented that mosaic of the Irish potato is a transmissible disease. In view of the fact that a large number of the experiments establishing the transmissibility of this disease were conducted in the greenhouse, it was considered advisable to confirm those results under field conditions. Furthermore, in connection with these experiments in the field additional contributions to our knowledge of mosaic of potatoes were secured. It will be the purpose of the following pages to present these results, which, unless otherwise indicated, have been obtained in northern Maine.

TUBER TRANSMISSION

MODIFICATION OF SEVERITY FROM YEAR TO YEAR

It is well known² that mosaic of Irish potatoes (*Solanum tuberosum* L.) is transmitted from one generation of plants to another through the tubers. It has been shown² that there may be great variation in the severity of the symptoms shown by the progeny of a given stock, strain, hill, or tuber.

Progeny of hills which appeared healthy during 1918 while growing in plots which contained some mosaic hills and which were situated near all-mosaic plots were grown and observed during the season of 1919. Most were of the Green Mountain, some of the Bliss Triumph, and a few of the Irish Cobbler variety. Each of the various lots contained some mosaic hills, the percentage varying from 12 to 76. Altogether there were over 4,000 hills, of which 1,200, or 30 per cent, were mosaic. In view both of results reported previously² and of the abundance of aphids in 1918, it seems that these mosaic hills represent cases of tuber transmission following aphid transmission occurring so late in the season of 1918 that no symptoms were apparent. The severity of the symptoms

¹ Conducted as one of the cooperative projects between the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, United States Department of Agriculture, and the Department of Plant Pathology of the Maine Agricultural Experiment Station.

² SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. M., and HAWKINS, Lon A. INVESTIGATIONS ON THE MOSAIC DISEASE OF THE IRISH POTATO. In Jour. Agr. Research, v. 17, no. 6, p. 247-273, pl. A-B, 25-30. 1919. Literature cited, p. 272-273.

shown by the diseased hills of any lot averaged either "slight plus," "medium," or "medium plus," although it was usually "slight" for many hills and often "bad" for some. "Slight" indicates characteristic mottling sufficiently rare to require careful search; "slight plus" means that mottling is readily apparent but is unaccompanied by wrinkling, "medium" represents both conspicuous mottling and some wrinkling, becoming "medium plus" with marked ruffling and more or less dwarfing; "bad" stands for extreme ruffling and dwarfing which may sometimes cause the mottling to be obscured.

Another similar series of small lots was grown in a second plot. In these the percentage of mosaic hills varied from 4 to 63, being 40 per cent for the 800 hills altogether. The severity of the symptoms shown by the diseased hills was about the same as for the lots in the first plot.

In addition to the healthy hill selections described above, stocks were grown from hills that showed mosaic in 1918. These contained 1,100 hills, of which only 5 had not yet shown mottling by July 30. These 5, of which 4 came from one tuber, were not observed later. It is possible that this healthy tuber, supposedly from an Irish Cobbler hill with bad mosaic, was formed by a long rhizome of a neighboring healthy hill, such as is seen occasionally, and was included with the tubers of the mosaic hill in spite of the precautions usually taken. The severity of the symptoms in the mosaic stocks is indicated in Table I.

TABLE I.—*Comparison of mosaic stocks in 1918 and 1919*

Variety.	Number of hills.		Severity of symptoms, 1918.	Severity of symptoms, 1919.	
	1918.	1919.		Variation between hills.	Average.
Green Mountain.....	25	204	Slight.....	Slight to slight plus....	Slight.
Bliss Triumph.....	34	269do.....	Slight to bad.....	Do.
Green Mountain.....	50	400	Medium.....	Slight plus to bad....	Medium.
Bliss Triumph.....	18	112	Bad.....	Medium plus to bad...	Medium plus.
Green Mountain.....	20	77do.....do.....	Bad.
Irish Cobbler.....	17	65do.....do.....	Do.

Evidently there was, in the stocks described in Table I, a tendency for the disease to change very little in severity as a result of transmission through the tubers from 1918 to 1919.

Two larger plots, one Green Mountain and one Bliss Triumph, were planted with stock from plots entirely mosaic in 1918. While the percentages of mottled plants on July 9 were, respectively, 67 and 89, all plants were mottled by the last of July. Although in magnitude the plants and yield were inferior to those of comparatively healthy lots, the appearance of the plants and of the plot as a whole was no worse than for the same stock during the three previous seasons.

The foregoing results indicate that mosaic in northern Maine does not necessarily change much from year to year in any diseased stock after the first appearance of the effects of infection. The conditions which determine the severity of the initial symptoms are not yet understood.

RELATION TO NUMBER OF TUBERS IN A HILL

The tubers from 10 Bliss Triumph hills and 130 Green Mountain hills, healthy in 1918 but grown near to diseased hills, were all planted uncut in hill lots in 1919. In Table II these hill lots are classified according to the number of tubers in a hill, and the percentage of tubers of each class that transmitted the disease is given.

TABLE II.—*Relation of the number of tubers in a hill to mosaic transmission*

Number of tubers per hill.....	2	3	4	5	6	7	8	9	10	11	12
Number of tubers planted.....	14	27	80	90	180	161	136	81	20	33	12
Percentage of tubers mosaic.....	86	60	32	53	38	46	46	41	30	36	41

There is a high percentage for the classes with two or three tubers to a hill, but otherwise no consistent relation obtains between number of tubers and percentage of mosaic. The results are not modified appreciably if the Bliss Triumph hill lots are disregarded. It thus seems that the increase of mosaic could be reduced by the selection of hills according to yield only if the hills with very low yields were discarded.

RELATION TO RELATIVE SIZE OF TUBERS

In connection with the problem of control, the question has arisen whether the selection of tubers according to size would have any effect in regard to the increase of mosaic. Consequently each of the 140 hill lots which are considered in the preceding section was planted in the order of decreasing apparent size of the tubers. With regard to mosaic 69 were mixed—that is, with both mosaic and apparently healthy plants in the same hill lot. In Table III the tubers of mixed lots are classified according to their relative rank, No. 1 being the largest. In addition, the percentage of tubers of each class that transmitted the disease is indicated.

TABLE III.—*Relation of the relative size of tubers in a hill to mosaic transmission*

Rank of tuber in size.....	1	2	3	4	5	6	7	8	9	10	11
Number of tubers planted.....	69	69	67	65	54	43	29	14	8	3	1
Percentage of tubers mosaic.....	67	48	42	46	44	42	38	36	38	33	0

The percentage is high for the group of tubers consisting of the largest ones in the hills and tends to decrease, being 48 per cent for No. 2, 45 per cent on the average for No. 3 to 6, and 36 per cent on the average for No. 7 to 10.

Another way in which to interpret the results is to consider all tubers of a hill lot as occupying equal parts of a line and to determine the "center of disease," which is the point on the two sides of which there are equal numbers of diseased and, if also possible, of healthy tubers. This center of disease was found, for the 69 hill lots described above, to be on the average closer to the large-tuber end of the hill-lot line, 44 per cent of the line being between the two. That is, there was a greater tendency to show mosaic as the relative size of the tuber was greater. However, this tendency is not marked enough to make it seem desirable to experiment further by selecting tubers according to absolute weight or size.

Of 357 hill lots planted in another plot, only the 2 to 6 largest tubers of each were planted, in order of decreasing apparent size. On July 22 to 26, 98 of the hill lots were mixed—that is, partly affected with mosaic. The results are similar to those given in Table III, the percentages being 57, 44, 48, and 35, respectively, for groups 1, 2, 3, and 4. The average center of disease is 46 per cent of the distance from the large-tuber end of the hill-lot line. Before this, on July 2 to 14, only 42 hill lots were mixed; and later, on August 22 to 25, a number of hill lots were either dead or too mature to show mosaic distinctly.

RELATION TO POSITION OF SEED PIECE IN THE TUBER

On July 29, 1918, 18 tuber units were observed which had been planted with quartered tubers and were mixed. Of the hills from stem-end quarters, 45 per cent were mosaic, while 62 per cent of those from bud-end quarters were diseased. Likewise there were 24 mixed tuber units of six plants each. Of the hills from stem-end sixths, middle-part sixths, and bud-end sixths, mosaic hills constituted, respectively, 43, 54, and 61 per cent. No attempt was made to sterilize the knife used to cut the tubers.

In 1919 each tuber was cut by means of one of several knives used in rotation and kept, when unused, with blades immersed in 4 per cent formaldehyde solution. Observations made June 28 to July 14 disclosed 44 tuber units, out of 1,109 observed, to be mixed. In these, 48 per cent of the plants from stem-end quarters and 51 per cent of those from bud-end quarters were mosaic. This slight difference had become more marked at the time of the next observation on July 22 to 26, when 84 tuber units, out of 1,348 observed, were mixed. At that time 28 per cent of the plants from stem-end quarters were mosaic, while 61 per cent of those from bud-end quarters were diseased. This difference was reduced slightly when it was found on August 22 to 25 that 20 more of the tuber units were mixed. The preponderance of mosaic in bud-end hills is of no value in the problem of control because of the small percentage of tuber units that are mixed. Its cause is not understood.

CONCLUSIONS REGARDING TUBER TRANSMISSION

Tubers from mosaic hills may be expected to transmit mosaic. In addition, at least part of those from apparently healthy hills growing near diseased plants will transmit the disease; and they tend to do so more when the parent hill contains only two or three tubers, when the relative size of the tuber in the parent hill is greater, and when the seed piece is nearer the bud end. However, hill selection results in discarding the hills with few tubers. The relation of relative size to mosaic transmission is not sufficiently marked or consistent to justify attempting tuber selection for the elimination of mosaic.

TRANSMISSION BY GRAFTING

TUBER GRAFTS

Grafting was attempted with a few tubers by bringing into contact the freshly cut surface of half a mosaic tuber and half a tuber from an apparently healthy hill. In 14 cases the nongrafted half of the supposedly healthy tuber remained healthy, and in 3 of these 14 cases the corresponding grafted half produced mosaic shoots. The three cases of apparent transmission were the only ones of the attempted grafts which established organic union. The failure of transmission in the 11 other cases indicates that mere proximity in a hill was not sufficient for transmission. Furthermore, the small number of successful grafts apparently was due to the fact that relatively old tubers were used.

DISEASED SCIONS UPON HEALTHY STOCKS

Since transmission by grafting had been somewhat effective both in the field with insects uncontrolled and in the greenhouse with insects controlled,¹ the same method was finally used in the field with insects excluded by means of cages. Three tuber units were used, each consisting of three hills. The untreated plants, the first hill of each unit, remained healthy until dug. In each other hill two or three stalks, from 14 to 17 inches high, were cut down and split, mosaic scions inserted, and contact established with the help of cord and adhesive tape. Soon after the dates of grafting, June 28 and July 2, 1919, the scions died because of shading in the cages; but the branches of the stocks made good growth, and by July 28 a branch in each of two grafted hills was mottled. By August 9 a number of shoots in each cage were mottled and were tagged. At the time of harvest, August 26, these were found to belong to the grafted hills. Healthy stalks also came from these hills but were ungrafted, one even coming from the same seed-piece eye as a grafted stalk.

As the Irish Cobbler variety had not been used for this kind of grafting, six mosaic scions were grafted upon uncaged stalks when the latter were

¹ SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. M., and HAWKINS, Lon A. OP. CIT.

6 inches high, on June 25, 1919. One scion died immediately, and the hill remained entirely healthy. In the other cases branches from the grafted stalks showed mosaic dwarfing with wrinkling and streak necrosis and some slight mottling in the leaves, while the nongrafted ones remained healthy.

TRANSMISSION WITH PLANT JUICE

STOCKS TREATED IN 1918

Although several methods of artificial inoculation performed in 1918 apparently had no effect,¹ the high percentages of mosaic shown by some of the 1919 progeny of the treated plants indicate that certain methods were effective. Of 76 plants, progeny of control plants treated with water, 24 per cent were diseased, probably because of aphid transmission in 1918; and of 463 plants, progeny of inoculated plants, 38 per cent were diseased, most of them probably because of aphid transmission. Of one lot of 53 hills, 77 per cent were mosaic. Those developed from progeny of plants which in 1918 were inoculated by means of capillary glass tubes inserted into the petioles immediately after these capillary tubes were taken from a similar position on diseased vines. All of another lot of 28 hills were mosaic. These were progeny of plants whose stems were split and partly immersed for several days in the juice expressed by crushing the tubers of mosaic plants. These two methods may be regarded as promising effective transmission if used in more extensive trials.

STOCKS TREATED IN 1919

In view of the fact that mosaic of potato was transmitted by transferring juice from diseased plants to the rubbed and crushed leaves of healthy plants first under greenhouse conditions,¹ it was considered advisable to confirm these results with a larger number of plants and under field conditions. Consequently, during the season of 1919 a series of similar inoculation experiments was conducted in field experimental plots, both in the open and under insect cages.

INOCULATIONS WITHIN THE SAME VARIETY IN THE OPEN

The first inoculation was made when the plants had reached a height of from 3 to 8 inches. The juice was expressed from the vines in a grinder and was separated at once from the pulp by straining through cheesecloth. At each treatment the undiluted juice was applied to the leaves after they had been bruised with the fingers. At each inoculation the controls were treated with juice from healthy vines before the plants to be treated with juice from mosaic vines were operated upon. One set of

¹ SCHULTZ, E. S., FOLSOM, DONALD, HILDEBRANDT, F. M., and HAWKINS, LOU A. OP. CIT.

instruments was used for the controls and another for the virulent juice. In these experiments the Green Mountain, Bliss Triumph, and Irish Cobbler varieties were used. In each case the juice was taken from vines of the same variety.

The plants of the Green Mountain and Bliss Triumph varieties used in this experiment developed from progeny which in 1918 showed from 11 to 15 per cent of mosaic, eliminated in three roguing. In view of the fact that, with the exception of the Irish Cobbler variety, these were planted by using four seed pieces from a tuber, it was possible to inoculate two of the hills in a tuber unit and have two additional hills of the same tuber unit remaining as uninoculated controls. In each tuber unit the plants in the second and third hills were inoculated—that is, a hill from a stem-end quarter and one from a bud-end quarter. In Table IV are given the results of these inoculations.

From Table IV it is apparent that plants not infected in 1918 if treated with juice from healthy vines remained healthy to the end of the season. (See Pl. 49-51.) As indicated, the exceptions to this result, where some tuber units produced plants which became mottled with mosaic after being treated with juice from healthy plants, were due to the fact that such units had become infected in 1918 in the field but did not present any evidence of infection at the time of the first treatment in 1919.

Plants inoculated with juice from mosaic-diseased vines showed the first mosaic mottling upon the newly developed leaves July 14. At this time aphids were just beginning to appear at the rate of a few individuals to a plant, so that those agents of dissemination can be disregarded as a factor in transmission in these open-field inoculations. It will be noted that with virulent juice a certain number of tuber units showed mottling throughout within a few days after the first inoculation, indicating that the tubers had become infected in 1918. In the remaining inoculated hills every hill, with the exception of one of Bliss Triumph, showed distinct mosaic mottling, while the untreated hills of these same units remained healthy to the end of the season.

In addition to the mosaic mottling, distinct spotting and streaking of the leaves, petioles, and stems obtained by July 25, so that at this time some of the lower leaves began to die. Furthermore, a marked ruffling and dwarfing of the leaves also became apparent, so that many of the plants appeared like those in the medium plus or bad stage, indicating that in a single season plants may develop an aggravated form of this disease if inoculated properly. (Pl. 52.)

TABLE IV.—Inoculations with juice from plants of the same variety

Variety inoculated.	Source of juice.	Date of inoculation.	Number of tuber units.	Noninoculated hills.		Inoculated hills.			
				Total number.	Number mosaic due to 1918 infection.	Total number.	Number mosaic due to 1918 infection.	Not mosaic due to 1918 infection.	
								Total number.	Percentage mosaic due to 1919 inoculation.
Green Mountain.....	Healthy Green Mountain.....	June 20 and 27; July 5 and 12.....	5	10	4	10	4	6	0
Bliss Triumph.....	Healthy Bliss Triumph.....	do.....	5	10	3	10	3	7	0
Irish Cobbler.....	Healthy Irish Cobbler.....	June 25; July 5 and 12.....	8	0	8	0	8	0
Do.....	Mosaic Irish Cobbler.....	do.....	12	0	12	100
Green Mountain.....	Mosaic Green Mountain.....	June 20 and 27; July 5 and 12.....	10	20	8	20	8	12	100
Bliss Triumph.....	Mosaic Bliss Triumph.....	do.....	10	20	8	20	8	12	91
Irish Cobbler.....	Mosaic Irish Cobbler.....	June 24, one inoculation only.....	12	0	12	100

Application of Juice from Plants Showing the Bad Stage of Mosaic

In order to determine whether juice taken from plants badly diseased with mosaic and introduced into healthy plants would induce bad mosaic symptoms in the latter, plants of the Green Mountain, Bliss Triumph, and Irish Cobbler varieties were inoculated in the same manner as those mentioned in Table IV. Three applications at weekly intervals were made upon plants of the same variety as that from which the juices were expressed. The height of the vines at the time of the first inoculation varied from 2 to 8 inches. Plants of five Green Mountain hills, three Bliss Triumph hills, and five Irish Cobbler hills were treated. At the same time also two Green Mountain, three Bliss Triumph, and two Irish Cobbler hills were treated with but a single inoculation.

On July 28, 16 days after the first treatment, the first mosaic mottling was noted upon the inoculated Irish Cobbler vines. By August 15 every inoculated plant, regardless of variety, showed distinct mosaic mottling as well as streaking and ruffling of the leaves as in the bad stage of mosaic; and by August 28 most of the leaves on the lower half of the stems were dead. The plants subjected to but a single inoculation showed symptoms similar to those given three successive treatments, indicating that a single treatment may be sufficient to induce the disease (Pl. 56).

INOCULATIONS WITHIN THE SAME VARIETY UNDER INSECT CAGES

Early Repeated Application

Juice from crushed mosaic plants (not necessarily mottled at the time of the first inoculation but from stock all mosaic in 1918) was applied to the bruised leaves of two hills in each of three caged tuber units on June 13, 20, and 27, and on July 5. As a control, the third hill in each cage was left untreated; also juice from apparently healthy plants was applied to the bruised leaves of two hills of each of three other caged tuber units on the same dates. In all these cases the plants were from 1 to 6 inches high at the first treatment. On July 9 the topmost leaves of the treated hills in the former three units began to show mottling, which was slight to medium by July 15. On July 30 mosaic branches in these units were tagged and were found at digging time, August 26, to belong to the treated hills, which had no healthy stalks. The tuber units upon which the juice from healthy plants was used remained green and healthy until digging time, while those died which became mosaic.

Late Application

On July 14 two hills in each of two caged tuber units were treated with juice from mosaic plants in the same manner as those described in the two preceding sections. Before August 20 the upper leaves of the treated hills became mottled and streaked.

INOCULATIONS FROM ONE VARIETY TO ANOTHER IN THE OPEN

Early Repeated Application of Juice

In order to determine whether the juice of a mosaic plant of one variety could induce the disease when introduced into a plant of a different variety of potato, intervarietal inoculations were made under open field conditions. The procedure of inoculation practiced in this connection was similar to that followed with the inoculations indicated in Table IV. In this experiment the control plants always were treated before mosaic juice was used, and a separate set of unstruments was employed for each distinct variety and for juice from each source. Green Mountain, Bliss Triumph, and Irish Cobbler varieties were used. These were subjected to four successive treatments at weekly intervals, as indicated in Table V.

The results given in Table V show that mosaic juice from one variety of potato may produce the disease when introduced into the plants of another variety. In these inoculations the effect upon the treated plants was fully as severe as that obtained when juice was introduced into plants of the same variety; as explained in connection with Table IV. In fact, in many cases the inoculated plants behaved like those in the late or bad stage of the disease. (Pl. 53-55.)

From Table V it is apparent that a large percentage of the plants had become infected in 1918. In view of the fact that such tuber units did not show the mosaic mottling at the time of the first inoculation, when the plants varied in height from 2 to 8 inches, it was impossible to restrict inoculation to healthy units. However, in this connection it is interesting to note that the hills infected in 1918 and inoculated in this experiment showed the disease like the plants in the bad stage whenever the uninoculated control hills in the same tuber units showed but slight or medium infection, so that apparently inoculation with juice increased the severity of the infection which had resulted from transmission in the field the previous season.

Since a considerable number of the plants in this experiment apparently had become infected in 1918, the evident objection might be offered that, in the course of the inoculation, infectious juice was carried from diseased to healthy plants of the same variety and thus caused infection. This objection can be eliminated. Inoculations always were commenced at the same end of the plot and row, and hence the respective tuber units were operated upon in the same consecutive order. In all cases, with the exception of Bliss Triumph inoculations with mosaic Irish Cobbler juices, the inoculated hills of the tuber unit treated first in each of the different varieties became diseased while the uninoculated hills of this unit remained healthy during the course of the experiment. Furthermore, a number of mosaic tuber units, apparently infected in 1918, were among the controls, or the units treated with juice from healthy plants.

TABLE V.—Inoculations with juice from one variety to another

Variety inoculated.	Source of juice.	Date of inoculation.	Number of tuber units.	Noninoculated hills.		Inoculated hills.				
				Total number.	Number mosaic due to 1918 infection.	Total number.	Number mosaic due to 1918 infection.	Not mosaic due to 1918 infection.		
								Total number.	Per centage mosaic due to 1919 inoculation.	
Green Mountain.....	Healthy Bliss Triumph.....	June 20 and 27; July 5 and 12.....	5	10	4	10	4	6	0	0
Do.....	Healthy Irish Cobbler.....	June 25; July 5 and 12.....	5	10	6	10	6	4	0	0
Bliss Triumph.....	do.....	do.....	5	10	4	10	4	6	0	0
Irish Cobbler.....	Healthy Green Mountain.....	do.....	5	10	6	10	6	4	0	0
Do.....	do.....	do.....	12	0	12	0	12	0	0
Green Mountain.....	Healthy Bliss Triumph.....	do.....	9	0	9	0	9	0	0
Do.....	Mosaic Bliss Triumph.....	June 20 and 27; July 5 and 12.....	10	20	3	20	3	18	17	94
Bliss Triumph.....	Mosaic Irish Cobbler.....	do.....	5	10	6	10	6	4	4	100
Do.....	Mosaic Green Mountain.....	do.....	10	20	10	20	10	10	6	60
Irish Cobbler.....	Mosaic Irish Cobbler.....	June 25; July 5 and 12.....	5	10	6	10	6	4	4	100
	Mosaic Green Mountain.....	June 26, one inoculation only.....	12	0	12	0	12	12	100

In no case did any healthy units become infected even though they happened to be treated immediately after a diseased plant had been operated upon. This indicates that infection does not carry very readily from one plant to another by merely rubbing the leaves of one plant and subsequently practicing the same operation upon a neighboring plant.

Late Application

On July 12, 1919, six healthy Green Mountain hills representing three different tuber units were inoculated with juice from mosaic Irish Cobbler vines. A second application was made upon these same plants a week later, when the vines were in blossom.

On August 15 distinct mottling was in evidence on the upper leaves of the vines in each of the six treated hills, and by August 22 some of the leaves were dying in spots and streaks as in the bad stage of mosaic.

Inoculations similar to the foregoing were made July 20 upon the vines of four hills in as many separate tuber units of the Irish Cobbler variety with juices from mosaic-dwarf Green Mountain vines. The plants at the time of the first inoculation had just finished blossoming. By August 20 slight mottling was noted upon the upper leaves of the inoculated vines and also slight streaking of the leaves as in bad mosaic stages. The results in these experiments indicate that plants can be inoculated successfully at the time of blossoming and later, as well as earlier in their development. Also, as stated previously in connection with insect transmission, even though mottling may not be in evidence in the season when infection occurs, nevertheless such plants will not fail to show distinct mottling under favorable environmental conditions during the following season.

INSECT TRANSMISSION

GREENHOUSE EXPERIMENT WITH APHIDS

Green Mountain tubers furnished by C. I. Gilbert were used at Orono with aphids in a greenhouse experiment because they were expected to be disease-free. This stock was used later in two plots. One consisted of 70 tuber units, of which only 1 was diseased early, evidently as the result of infection in 1918. The other, grown and observed in southern Maine by Dr. W. J. Morse, consisted of 1,357 hills, of which less than three-fourths of 1 per cent were mosaic. In the greenhouse experiment 10 tubers were each cut lengthwise with a flamed knife into four sets and planted on March 17. Half the plants from each tuber were inclosed with insect cages, into each of which about 150 individuals of the common green peach aphid, or spinach aphid (*Myzus persicae* Sulz.) from mosaic potato plants were introduced on April 13 to 16, when the plants were from 2 to 9 inches high. To 15 plants aphids were introduced on leaves on a stick thrust into the soil so that they dispersed without contact between the diseased leaves and the treated plant. To 5 plants they

were introduced on terminal-shoot buds in a flask laid upon the soil. The aphids were killed by nicotine fumigation on April 21. All plants appeared healthy when observed by one of the writers on April 21, when from 10 to 25 inches high. Between April 21 and June 2¹ mosaic symptoms appeared on all of the 15 plants to which the aphids were introduced on sticks. Of the 5 plants to which the aphids were introduced in the flask only 1 became mottled, on July 8. When introduced in the flask many aphids had been injured or killed by water condensing on the interior of the flask following transpiration by the bud. Nineteen of the 20 untreated plants remained healthy; 1 showed slight symptoms on July 9. This plant was the only one found on or before April 28 with uncontrolled aphids upon it—possibly from a mosaic plant or a plant treated with virulent aphids. It was again found to be infested on May 19 and 26. In the case of the 15 plants treated with the stick method of introducing aphids, the percentage showing infection and the average length of the period between treatment and the appearance of the symptoms were greater than in the case of plants treated similarly in a previous greenhouse experiment,² probably because in the later trial the plants elongated to heights of from 44 to 72 inches and thus offered for a longer period a chance for the initial display of mottling in the young leaves.

FIELD EXPERIMENTS WITH CAGES

EFFECT OF THE USE OF CAGES IN 1918

Although the cages for the control of insects in 1918 did not inhibit completely the dispersal of aphids, nevertheless their use materially checked transmission of mosaic. The effect of these cages upon transmission of mosaic is indicated in Table VI.

TABLE VI.—Effect of cages on transmission of mosaic

Variety.	Number of hills selected in 1918.	Number of tubers selected for 1919 planting.	Treatment in 1918.	Percentage of mosaic in 1919.
Green Mountain.....	9	32	Uncaged.....	49
Do.....	3	6	Caged with mosaic hill.....	100
Do.....	22	50	Caged.....	0
Bliss Triumph.....	31	66	Uncaged.....	35
Do.....	20	54	Caged.....	0

The number of hills reported in Table VI includes only a small percentage, a representative lot, of the total number planted in 1918. However, each hill indicated was grown under a separate cage. While these

¹ Observations after May 1 were made weekly by Viola L. Morris, laboratory assistant, and finally by Dr. W. J. Morse, neither having any information regarding the previous treatment of any plant.

² SCHULTZ, E. S., FOLSOM, DONALD, HILDEBRANDT, F. M., and HAWKINS, LON A. OP. CIT.

results might be interpreted as suggesting that some insect besides aphids was a deciding factor, it is possible for the aphids observed in the cages late in the 1918 season to have come from a very few which did not carry mosaic, and as yet no other insect is known to transmit mosaic of potato.

EXPERIMENTS WITH APHIDS

Small colonies of the peach aphid were brought from Orono May 1 on radish and mosaic potato plants. Both increased while feeding on these plants. On June 7, when the vines were from 1 to 4 inches high, 9 caged plants of 3 tuber units were treated with aphids from radish plants, about 150 to each hill. These 3 units were regarded as controls, since the aphids had lived for a number of generations on radish plants and were supposed to be free from a mosaic virus. Three caged plants of a fourth tuber unit were treated with aphids from a mosaic potato plant; 2 plants were left in each hill and the aphids, about 100 to a hill, were introduced on leaves on a stick thrust into the soil near each hill. On June 30 and July 5, when aphids were very numerous, these 12 plants were sprayed with a solution of soap and nicotine sulphate. The plants used came from the Gilbert stock, already described as exceptionally healthy. On July 28 the fourth tuber unit was slightly mosaic in some branches of 1 hill, and by August 9 it was dead, as the result of excessive aphid infestation. The 3 controls remained healthy until dug on August 26.

On June 17, nine half-tuber sets from stock caged in 1918 were planted under three cages. On June 28, when the vines were from 1 to 3 inches high, the plants were treated with aphids from mosaic plants; several hundred aphids were introduced by each hill with the stick method described above. They were sprayed on July 5 and 8. On August 9 one hill showed some mosaic. When dug on August 26, this hill was all mosaic, while two other hills—one in the same cage—were each mosaic in the upper leaves of one stalk. The untreated plants from the other nine half tubers were grown in the field and remained healthy throughout the season.

Four tuber units of the Gilbert stock, comprising 12 hills, were treated on July 12, when the plants were large enough to press against the tops of the cages. The first unit was treated with hundreds of aphids from radish plants and the others with aphids from mosaic potato plants. In the latter case several thousand aphids were left on the diseased leaves and stems in a flower-pot saucer set at the base of each of the first and third hills, whence they dispersed within a few days. The first tuber unit remained healthy throughout the season. The other three were still healthy on August 9, but when dug on August 26 two hills were mosaic, each in the upper leaves of one branch of a stalk.

EXPERIMENT WITH FLEA BEETLES

Three caged tuber units (9 hills) of the Gilbert stock were treated with flea beetles (*Epitrix cucumeris* Harris) on June 13, 1919, when a few inches high. The middle hill of each unit was covered with a cylindrical cage set inside the larger cubical one; the other two hills were treated with several hundred flea beetles. These insects were collected from small potato vines which developed from 100 per cent mosaic stock. On June 20 the cylindrical cages were removed and most of the flea beetles, which had damaged the plants considerably, were driven out of the cages or killed by hand. On June 16 two more similar tuber units were treated likewise. All the hills remained healthy until dug on August 27.

As controls, four similar tuber units were treated in the same way, except that the beetles were taken from plots of mostly healthy potatoes or, in one unit, from bushes near the potato field. All the hills remained healthy until dug on August 26.

EXPERIMENT WITH COLORADO POTATO BEETLES

Five caged tuber units (15 plants) of the Gilbert stock were treated with Colorado potato beetles (*Leptinotarsa decemlineata* Say.) on July 3, 1919, when they reached nearly to the tops of the cages. The insects were gathered with brush and pan from plants in all-mosaic plots when from 2 days old to two-thirds full grown. Two stalks were left in a hill, and the first and third hills in every cage were treated with over 100 of the larvæ each. These were shaken from the gathering pan upon a cloth and were either rolled upon the leaves or left on the cloth while it was laid on the plant. Within 24 hours the plants had been damaged rather severely. They were sprayed with an arsenical poison, which soon caused the death of the larvæ. All the plants remained healthy until dug on August 26 and 27.

Three similar tuber units were treated likewise on July 7, except that the larvæ were obtained from plants in plots almost disease-free. These also remained healthy until dug on August 27.

FIELD OBSERVATIONS WITHOUT CAGES

GREENHOUSE STOCKS

Tubers from the 53 plants used in the first aphid experiment performed in the greenhouse at Orono¹ were planted whole. All of the 37 tubers from plants which became mosaic after the introduction of aphids from mosaic potatoes produced diseased hills, except 2 which came from a plant with 3 out of 7 stalks apparently healthy. The 2 healthy tubers were probably produced by the 3 healthy stalks. All of the 10 tubers from plants which remained apparently healthy until harvested, although they were fed upon by aphids from mosaic plants, were

¹SCHULTZ, E. S., FOLSOM, Donald, HILDERBRANDT, F. M., and HAWKINS, Lon A. OP. CIT. p. 25-30.

mosaic. None of the 38 tubers from caged untreated plants or of the 15 from plants fed upon by aphids from a healthy potato plant were mosaic. Of the 34 tubers from uncaged and untreated plants 1 was mosaic; it came from a half-tuber hill that early showed ruffling and chlorosis along the veins but no typical mosaic mottling such as was shown, in addition to these incomplete symptoms, by the corresponding half-tuber hill after treatment with virulent aphids. Of the 37 tubers from plants fed upon by aphids from radish plants 4, or 11 per cent, were mosaic; these 4 came from 2 plants recorded as having been fumigated to eliminate a few aphids which were found on them and which were of unknown origin, possibly from neighboring diseased plants.

These results agree essentially with those which were secured previously with the first generation of the same stocks and which were described to prove the possibility of transmission by aphids. They also indicate that (1) mosaic mottling may be restricted to the parts of the leaf along the veins, (2) a plant with three stalks healthy and four mosaic may produce three mosaic tubers and two healthy ones, thus explaining the partial infection of hill lots, (3) plants treated with virulent aphids may appear healthy but produce progeny that are all mosaic, as shown previously by the writers,¹ and (4) apparently healthy plants inspected often for aphids and fumigated to eliminate these insects as soon as they are discovered may produce progeny of which a small percentage is mosaic.

In connection with the experiment just considered it was necessary to treat a number of control plants by laying a mosaic leaf upon each. These were kept in a different greenhouse room where aphids were more abundant, and they were never caged. Of 45 tubers from these, and also of 25 tubers from similar plants with no leaf laid on, 20 per cent were mosaic, all coming from plants recorded as being fumigated to eliminate uncontrolled aphids found upon them.

PROXIMITY STUDIES WITH PLOTS

In 1918, plots 1, 2, and 3 were each rogued of mosaic hills three times. Stocks from the first two, Green Mountain and Bliss Triumph, respectively, each showed mosaic in 20 per cent of the hills in 1919, while that from No. 3, Green Mountain, next to No. 4, a Green Mountain plot with 45 per cent of the hills diseased, showed mosaic in 30 per cent of the hills. In 1919, each of the stocks was rogued several times and grew between similar stocks. All these plots in both years were each $\frac{1}{4}$ acre in area. The greater percentage of mosaic in 1919 in stock from plot 3 can be explained best by the greater proximity in 1918 to a half-mosaic plot and by consideration of the apparently greater ease of dispersal of aphids, which were numerous in 1918, from the half-mosaic plot to No. 3. Plots 1, 2, and 3 were planted with stocks A, B, and C, respectively, described in Table VII.

¹SCHULTZ, E. S., FOLSOM, DONALD, HILDEBRANDT, F. M., and HAWKINS, LOU A. OP. CIT.

INTERSEASONAL INCREASE

It was very apparent that aphids, which seemed as abundant as the flakes in an ordinary snowstorm when they were migrating in the late summer, were unusually numerous in 1918. Consequently it is of interest to compare the relative interseasonal increase in mosaic in the same stocks from 1917 to 1918 with that from 1918 to 1919. It has been demonstrated in several greenhouse experiments already discussed that aphids may transmit mosaic without the symptoms being shown until the progeny of the inoculated plants is grown the following season. It is considered that they may do likewise in the field during the latter half of the summer, which is usually the only time when they are abundant on potatoes in northern Maine, although there are more species in the field than were used in the various experiments.

Previous experiments seemed to indicate that the percentage of mosaic in susceptible varieties could be materially reduced by roguing the diseased plants from the plots as soon as the mottling appeared upon the vines. However, before it was fully demonstrated that insects were capable of transmitting mosaic the plots usually were arranged in such a manner that insect transfer could take place very readily. In view of this situation, it is possible to note the effects of those agents of transmission upon the performance of a few of the plots, each including ¼ acre, which were rogued during the last three seasons. Table VII records the observations on these plots.

TABLE VII.—Relation of aphids to increase of mosaic from season to season

Variety.	Stock.	1917.			
		Location.	Per-centage of mo-saic.	Treatment.	Number of aphids.
Green Mountain...	A	Next to 100 per cent mosaic stock..	32	Rogued three times...	Few.
Bliss Triumph....	Bdo.....	44do.....	Do.
Green Mountain...	Cdo.....	49	Rogued once.....	Do.

Variety.	Stock.	1918.				1919.
		Location.	Per-centage of mo-saic.	Treatment.	Number of aphids.	Per-centage of mo-saic.
Green Mountain ..	A	Six rows from 45 per cent mosaic stock.	11	Rogued three times.	Very abundant .	20
Bliss Triumph....	B	Nine rows from 45 per cent mosaic stock.	16do.....do.....	20
Green Mountain..	C	Next to 45 per cent mosaic stock.	13do.....do.....	30

It will be noted in Table VII that in 1917 certain factors seemed to be more favorable for the spread of mosaic than in 1918—namely, higher percentage of diseased hills (rogued) in the plots, greater proximity of unrogued mosaic stock, and higher percentage of mosaic in the nearest unrogued diseased plot. However, there was less spread in 1917 than in 1918, as shown by the lower percentage of mosaic in 1918 than in 1919, in correlation with the greater abundance of aphids in 1918. Furthermore, these observations indicate how difficult the problem is of producing perfectly mosaic-free stocks from susceptible varieties wherever these agents of transmission exist.

EFFECT OF VARIATION IN THE TIME OF HARVESTING IN 1918

It was expected that if aphids were a deciding factor in mosaic transmission the lots of tubers harvested at progressively later dates during their increase in numbers would show an increasing percentage of mosaic. Seventy-eight healthy hills (66 Green Mountain and the rest Bliss Triumph or Irish Cobbler) were selected in 1918 in a plot containing many small lots all with more or less mosaic. Aphids became noticeable on potatoes the last part of July and increased in numbers so that they were very numerous about the middle of August and more excessively abundant as the end of the month was approached. Tubers about an inch in diameter were harvested on August 8 but did not keep with the methods used. Another set of tubers was harvested on August 15 and a third on August 26, one tuber being removed from every hill on each date. On September 12 the remaining tubers—321 in all—were harvested. The tubers were planted uncut in 1919 and transmitted 6, 14, and 50 per cent of mosaic, respectively, for the three lots. Apparently some of the infection occurred before August 15, but most of it was too late to affect many of the tubers harvested by August 26. This difference can be explained best by the great increase of aphids during August, together with the results obtained in the experiments on aphid transmission.

TEST OF THE SEED-CUTTING KNIFE

In 1919 stock was available from 1918 all-mosaic plots and rogued plots. One hundred tubers from the former were divided by three parallel transverse cuts so that no two cut surfaces joined in a seed piece, while 100 tubers from the latter were quartered by a transverse and a longitudinal cut so that each seed piece had two cut surfaces joining at a right angle. The same knife was used, cutting alternately tubers from the two lots. The 800 sets were left mixed in the same sack for over a day and planted by hand at 15-inch intervals in two rows. Another mixture was prepared in the same way with 200 tubers from the same two barrels, but in this case the pieces from the all-mosaic lot were sorted

out and discarded and only the others were planted. The latter occupied the third row, and the fourth row was used for a control lot prepared similarly except that no all-mosaic stock was used. Upon examination of the four rows on July 23 the control row was found to contain 85 mosaic hills, the third row 72, and the first two 475—that is, 75 excluding the 400 from all-mosaic stock. No change in the number of mosaic hills was found on August 18. A $\frac{1}{4}$ -acre plot of the rogued stock was planted elsewhere and contained 80 mosaic hills in each 400. Evidently the furnishing of conditions apparently optimum for knife transmission had no effect upon the mosaic percentage.

It was thought in 1918 that the partial infection of tuber units might be due to knife transmission. As stated before (p. 318), in 1919 when tuber units were planted three knives were used in rotation, each one being immersed in a 4 per cent formaldehyde solution when not in use. However, the partial infection of tuber units and hill lots was as common as before.

TESTS OF EFFECTS OF CONTACT

GREENHOUSE EXPERIMENTS

As has been reported,¹ out of nine healthy plants kept in contact with mosaic plants in a greenhouse one showed mosaic, but not until after a few uncontrolled aphids, possibly from mosaic plants, were discovered upon it. At about the same time, March 13, 1919, each of 12 tubers was split into three sets and planted in small pots. The plants from 4 tubers became mottled by April 1 when from 3 to 13 inches tall. The other 24 were transplanted about April 1 into large pots, 2 from each tuber into steam-sterilized soil and the third into soil containing a mosaic plant. The transfer was made by knocking off the bottom of the small pot and setting it into a hole formed by a small empty pot put in when the mosaic set was planted. The method used permitted the mingling of the roots of the two plants while it kept the two sets of tubers mostly apart and facilitated harvesting them separately. The vines of the two plants were twisted and tied together. All of the 24 plants remained healthy until July 9.² They had ceased to elongate by this time and soon afterwards were dug. The tubers were not planted, because of the abundance of aphids on the plants in July.

FIELD EXPERIMENTS WITH INSECT CAGES

Nine tubers of the Gilbert stock were planted halved in 1919, each two sets being separated by a mosaic set and all three caged. On July 30 three of the mosaic hills were dead or nearly so. The Gilbert hills all remained healthy until August 9 and when dug on August 27

¹ SCHULTZ, E. S., FOLSOM, DONALD, HILDEBRANDT, F. M., and HAWKINS, LOU A. OP. CIT.

² Observations after May 1 were made weekly by Viola L. Morris, Laboratory Assistant, and finally by Dr. W. J. Morse.

were entirely healthy except for mosaic mottling in the few uppermost leaves of several branches of a stalk in one hill. These leaves appeared young. They had evidently been pushed hard against the inside of the cage and had a very few aphid skins and aphids clinging to them. They may have been infected as the result of contact before aphids entered the cage, by aphids on the outside of the cloth against which the leaves were pressed, or by aphids that came from mosaic plants in the next row and that entered through a small hole that was found to have been made accidentally in the cloth.

FIELD EXPERIMENTS WITHOUT CAGES

As was pointed out in a previous section regarding the test of the seed-cutting knife, the mixing of all-mosaic stock and rogued stock in two rows was not followed by a higher mosaic percentage for the rogued stock than was shown by it in a control row. The negative results in this case do not disprove the possibility of infection occurring too late to be evident during the current season—that is, after the roots and vines have become intertwined.

In 1918 five Green Mountain hill lots were found to be partly mosaic. The healthy hills were harvested separately, were classified according to their proximity in the row to a mosaic hill, and the tubers were planted uncut in 1919. Twenty-eight tubers were progeny of plants each of which grew between two mosaic hills, and 54 per cent of them were mosaic. Eighty-nine were progeny of hills each of which was between a mosaic hill and a healthy hill, and of these 63 per cent were mosaic. On the other hand, 40 per cent of the 220 tubers from hills each of which grew between two healthy hills were diseased. If these 220 tubers are arranged in five groups, H₁, H₂, H₃, H₄, and H₅, according to the increasing number of healthy hills between the parent and the nearest mosaic plant in the row, the groups contained, respectively, 75, 53, 41, 33, and 18 tubers, with 56, 24, 54, 24, and 17 per cent of them diseased. Since being next to a mosaic plant in the same row seemed to increase the chance of infection as much as 54 or 63 per cent is greater than 40 per cent, it evidently is a contributing factor in mosaic transmission; but judging from the varying percentages of infection among the classes of plants which were not next to mosaic hills in the same row, it probably aids in the spread of the disease only by aiding aphid transmission.

A slightly different type of experiment consisted in comparing the progeny of three small 1-row Green Mountain lots, of from 100 to 200 hills each, from which the mosaic hills (respectively, 6, 16, and 30 per cent) were removed on August 1, 1918, with two similar lots from which the mosaic hills (respectively, 6 and 18 per cent), together with each healthy hill next in the row to a mosaic one, were removed

August 1. In spite of the differences in contact with diseased hills, the progeny of the two lots were 27 and 35 per cent mosaic, respectively, and the progeny of the three lots were from 25 to 35 per cent mosaic. Aphid dispersal from neighboring mosaic plots was easy, and it apparently nullified any effect that the difference in contact might have had.

TEST OF SOIL HARBORING

GREENHOUSE EXPERIMENT

At harvesting time in 1918 one tuber was taken from each healthy hill in two hill-selected lots. At Orono on January 14, 1919, these tubers were split with a flamed knife, and one set was planted in steam-sterilized soil and the other in soil from which a mosaic plant had been removed on December 30 or January 13. Nineteen pairs of half tubers were used, and the plants from 7 pairs were mosaic by February 22, when from 1 to 20 inches high. The plants from the other 12 pairs reached their maximum height about March 5 and remained healthy until dug in April. The second generation of the 12 healthy pairs was grown and found to be entirely free from mosaic. It is clear that there was no transmission by the soil in which mosaic plants had just been grown, all mosaic that was shown evidently being transmitted by the tubers.

FIELD EXPERIMENTS

The greenhouse experiment described in the previous paragraph was not concerned with certain factors in the possible soil-harboring of mosaic in fields—namely, old stalks, volunteer potato plants, and insects. There is no doubt, when the proofs of transmission by aphids are remembered, but that volunteer mosaic plants may contribute to the infection of healthy stocks planted where mosaic stocks were grown the preceding season if they are not discovered and removed before the appearance of aphids. Even if they are, other factors might cause the infection of healthy plants.

To test this supposition, three rows of Green Mountain stock from a plot rogued in 1918 were planted across the location of a 1918 20 per cent diseased Green Mountain plot and a wholly diseased one. Each mosaic hill was dug and the seed piece examined. If volunteers are disregarded, 28 per cent of the 142 hills grown upon the ground of the all-diseased plot were mosaic as were 28 per cent of the 481 plants grown upon the ground which had produced the 20 per cent mosaic plots. This evidently was from infection the previous season, since 27 per cent of the hills were mosaic by July 15.

A similar but more extensive test consisted in planting 19 rows of the same stock across the ground which had produced 14 of the 1918 plots. Similar examination of the mosaic plants on July 30 showed 22 per cent

of the 4,466 hills to be mosaic. Although this stock was retarded in its development by being frozen nearly to the ground on June 23, only 1 per cent of the hills developed mosaic between July 30 and August 18. The nature of the various 1918 plots and the percentages of mosaic on the same ground in 1919 are given in Table VIII.

TABLE VIII.—*Nature of 1918 plots and percentage of mosaic hills in the parts of the 1919 plot grown upon the same ground*

Section No.	1918.		1919.	
	Variety.	Disease.	Total number of hills.	Percentage of mosaic hills from seed pieces.
1	Green Mountain.....	11 per cent mosaic.....	424	24
2	Bliss Triumph.....	15 per cent mosaic.....	432	23
3	Green Mountain.....	13 per cent mosaic.....	454	22
4do.....	45 per cent mosaic.....	422	26
5do.....	46 per cent mosaic.....	375	18
6	Roxbury Wilson.....	10 per cent mosaic.....	281	23
7	Bliss Triumph.....	100 per cent mosaic.....	350	23
8	Green Mountain.....do.....	458	22
9do.....	11 per cent mosaic.....	140	28
10	Irish Cobbler.....	No leafroll.....	143	22
11do.....	All leafroll.....	105	22
12do.....	No leafroll.....	169	15
13	Miscellaneous.....	Leafroll and mosaic.....	140	23
14do.....do.....	573	24

It will be noted that there are few marked deviations from the percentage for the whole plot, which was 23 per cent. These consist of one deviation upward and one downward for the ground occupied by two half-mosaic plots (4 and 5) and of the same for two comparatively mosaic-free plots (9 and 12) and therefore are without significance in regard to soil-harboring of the disease.

SUMMARY

(1) Transmission of potato mosaic by means of tubers, grafting, plant juice, and aphids was effected under various conditions, including those essentially of the field with insects controlled.

(2) Infection was obtained with intervarietal transfer of juice.

(3) Transmission was attempted, but without success so far as could be ascertained in the same season, by means of flea beetles, Colorado potato beetles, the seed-cutting knife, and contact of seed pieces, of roots, and of vines.

(4) Preliminary observations indicate that infection does not result from growth in soil that produced mosaic potato plants the previous season.

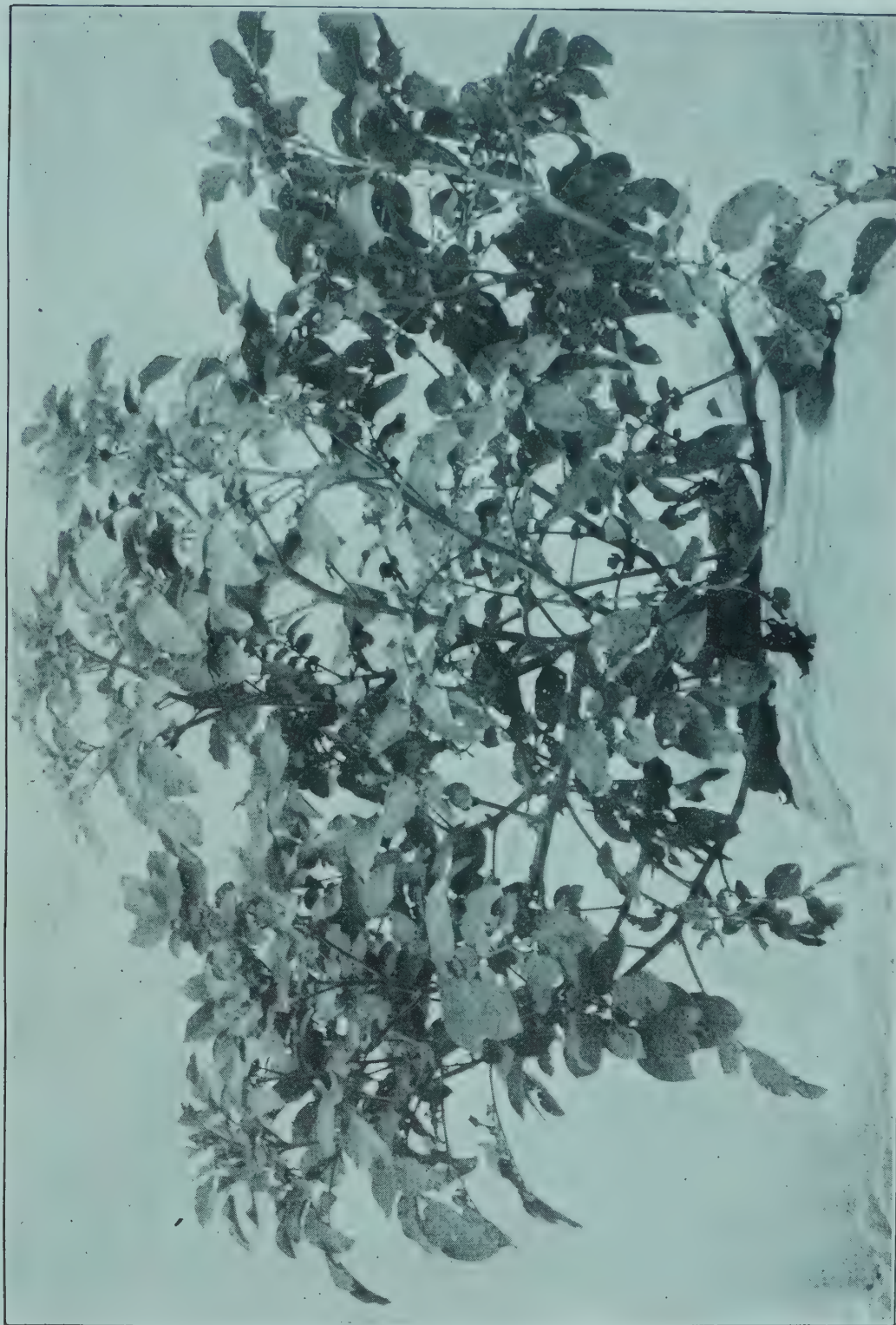
(5) It appears impossible either for infected plants to recover or, so long as diseased stock is not far off and insect carriers exist, to assure the maintenance of health of susceptible varieties by roguing plots or by selecting hills, tubers, or seed pieces.

(6) Isolation of plants by means of insect cages, as well as elimination of insects in the greenhouse, have maintained stocks disease-free, indicating that control of aphids and possibly of some other kinds of insects as well, is the most important means of checking the spread of potato mosaic among susceptible varieties.

PLATE 49

Vines of Green Mountain variety inoculated with juice from healthy foliage of the same variety. No mottling and no ruffling of leaves.

(338)



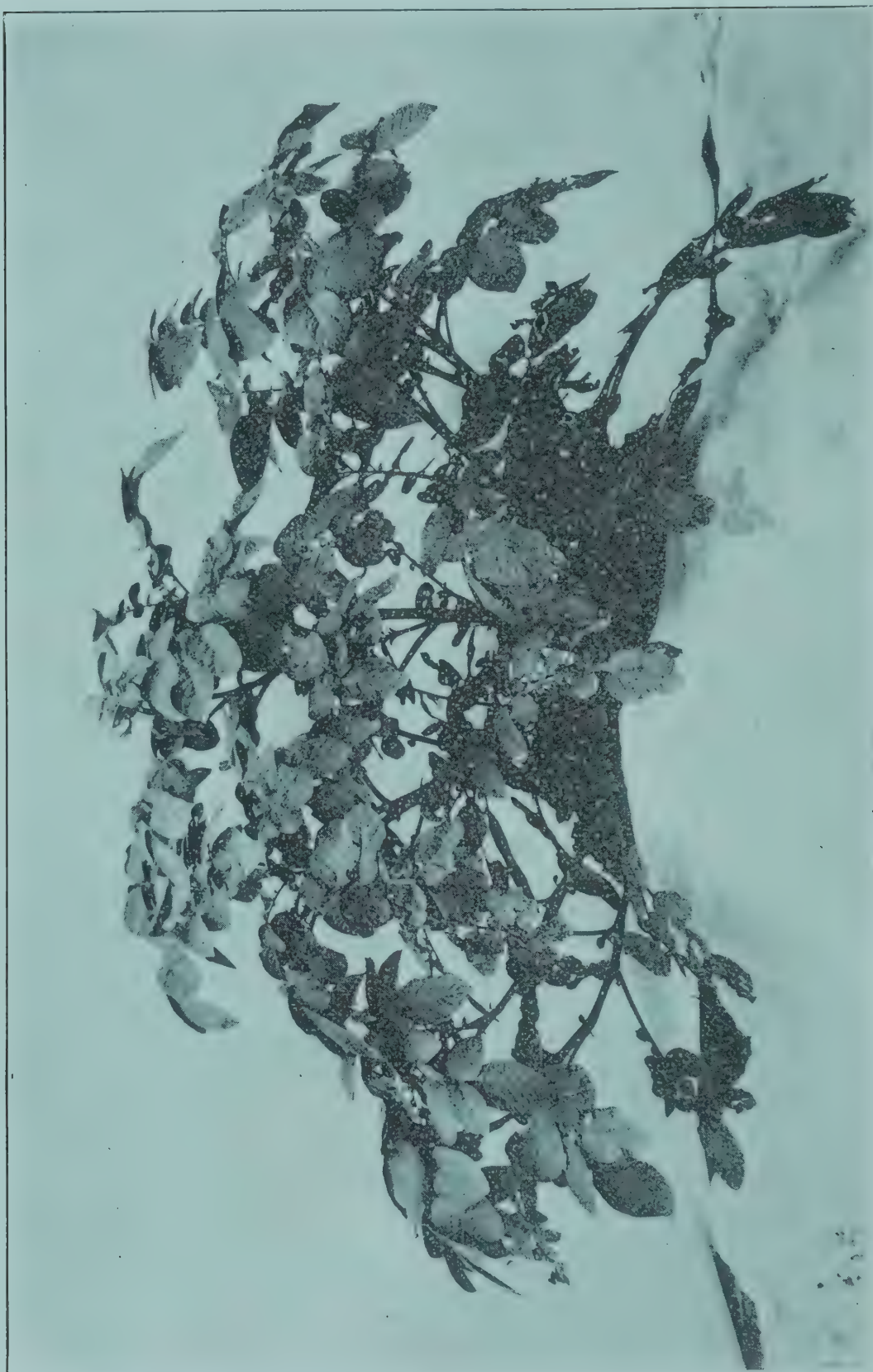


PLATE 50

Vines of Bliss Triumph variety inoculated with juice from healthy foliage of Irish Cobbler variety. No mosaic mottling.

PLATE 51

Vines of Irish Cobbler variety inoculated with juice from healthy foliage of the same variety. No mosaic.



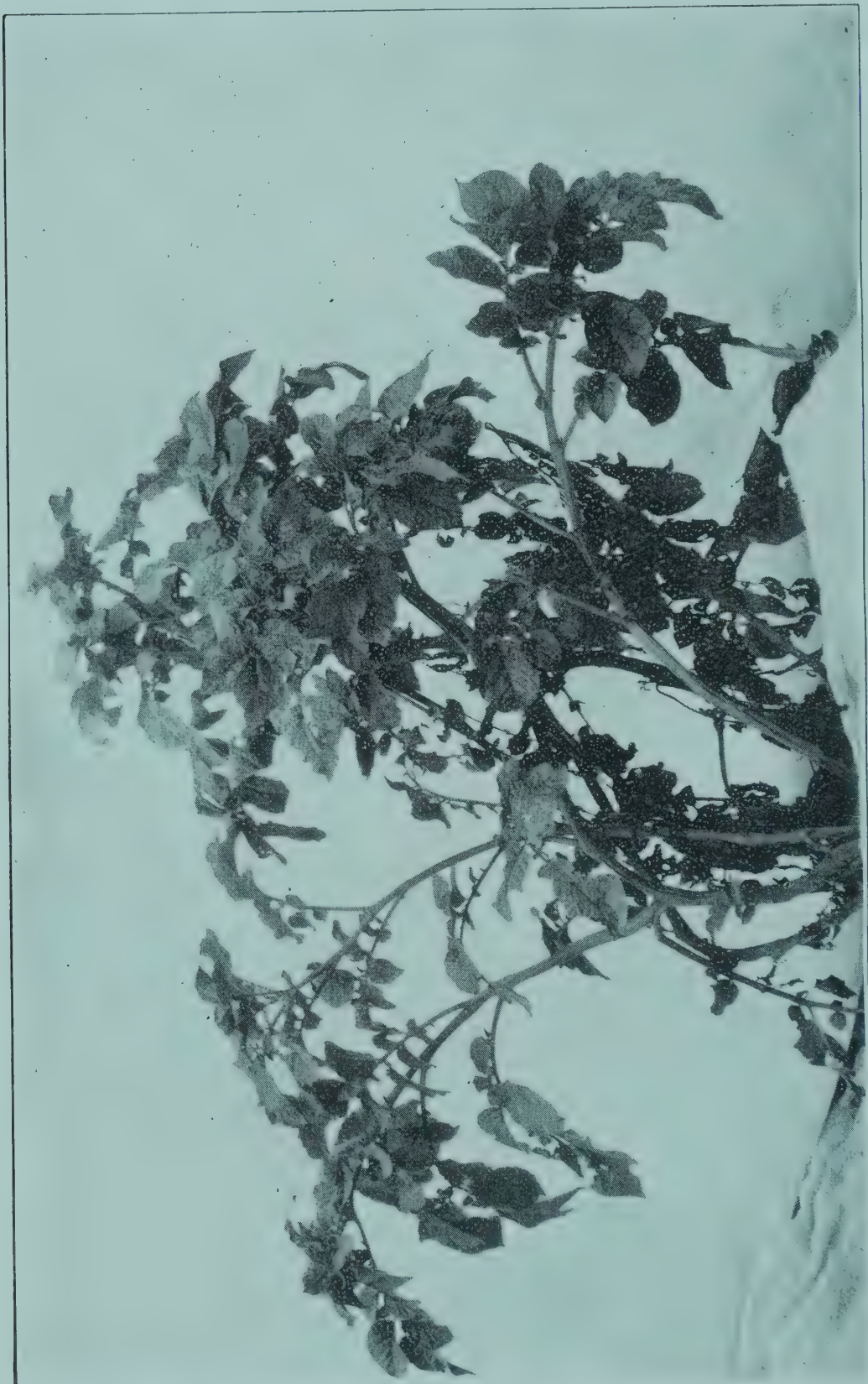


PLATE 52

Vines of Green Mountain variety inoculated with juice from mosaic foliage of the same variety. Distinct mosaic mottling and ruffling of young leaves on top of stalks. For control see Plate 49.

PLATE 53

Vines of Green Mountain variety inoculated with juice from mosaic foliage of Bliss Triumph variety. Distinct mottling and ruffling of upper leaves and early dying of lower leaves. Condition of control plants same as vines in Plate 49.





